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ADDRESSING VARIOUS CHALLENGES RELATED TO FOOD BOLUS AND NUTRITION WITH THE AM^2 MASTICATION SIMULATOR

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Abstract

Preparing a food bolus for swallowing is the first and major goal of oral processing ensured by mastication. To fulfill this goal, mastication is accurrately adjusted to the structure and texture of the food. Since the bolus is the main outcome of food oral processing, there is growing interest in improving understanding the food bolus and its features at the end of the masticatory sequence and throughout its formation. Although this step is trivial, its execution is somewhat more complex than it appears. Until now, the food bolus produced has not subjected to full analysis, mainly due to the lack of accessibility during *in vivo* oral processing.

To overcome this difficulty, we developed a masticator apparatus, named AM^2, on the basis of *in vivo* compression and shear stresses applied on food during oral processing. The apparatus was validated against particle size analyses of boluses produced by individuals presenting normal mastication. The present paper provides a comprehensive overview of the various potential uses of the AM^2 apparatus to produce food boluses for a large range of scientific needs.

The food bolus contains much valuable information regarding the progress of mastication, the completion of the function and its role in food disruption, the oral release of food compounds, changes and bioaccessibility, and finally its impact on nutrition. The AM^2 apparatus can also be used to simulate mastication in specific populations, such as the elderly and children, and to address various objectives related to this initial step of digestion.

Keywords

artificial mastication, food bolus, oral release, oral bioaccessibility, oral digestion, nutrition

Highlights

- The mastication simulator provides food bolus for many foods and various purposes.
- Food disruption, the oral release of food compounds, and digestion can be analyzed.
- Food bolus analysis provides pertinent knowledge on deficient mastication.
- AM^2 reveals *in vitro* bolus features (dynamics, formation) in various oral conditions.
- AM^2 is a very attractive model for mechanistic digestion/nutrition studies.

1. Introduction

Food oral processing and more specifically the ready-to-swallow food bolus have become major topics of interest in food and dental science (Engelen, Fontijn-Tekamp, & Bilt, 2005) and in the field of nutrition (Bonnet, Batisse, Peyron, Nicolas, & Hennequin, 2018; Chen, 2009, 2015; Engelen, Fontijn-Tekamp, & Bilt, 2005; Ng et al., 2017). Food bolus analysis is at the crossroads between food structure (Gao, Wong, Lim, Henry, & Zhou, 2015), food formulation, the oral perception of food, food oral processing (Hwang et al., 2012; Jalabert-Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Koç et al., 2014; Shiozawa, Kohyama, & Yanagisawa, 2003) and the further stages of digestion (Hoebler, Devaux, Karinthi, Belleville, & Barry, 2000; Hoebler et al., 1998), including oral digestion and related nutrient bioaccessibility (Ellis et al., 2004; Grundy et al., 2015). However, the mere fact that food bolus formation occurs inside the mouth and that access inside the mouth is not possible during mastication hampers most study designs. Collecting the ready-to-swallow bolus in normal or deficient subjects is not a simple procedure. Among other reasons, the bolus is

often partly swallowed before the final swallow event (Hiiemae et al., 1996), the bolus itself is mixed in an unknown proportion with saliva, making it impossible to determine the proportion of saliva, food fluids and solid nutrients present within the bolus during the masticatory process. During chewing, several physico-chemical processes are at work, transforming the characteristics of the final bolus in an uncontrolled way. In addition, asking volunteers to masticate is sometimes impossible for several reasons such as the use of "experimental" or non-natural food, the presence of food contaminants, the chewers studied are disabled, young children or elderly people, or simply because it is too time consuming. Therefore, *in vitro* mastication using a mastication simulator is a valuable alternative to an *in vivo* approach for the experimental production and analysis of food boluses. This kind of simulator also provides solutions for multiple analyzes of food bolus properties at the time of swallowing or of the kinetics involved during its formation, and it provides a more realistic oral simulation than that frequently ~~done~~ performed before (Funami, 2016; Hedrén, Diaz, & Svanberg, 2002; Hoebler et al., 2002; Minekus et al., 2014). The possible questions of interest include saliva impregnation, the oral release of nutrients and oral digestion assessment, investigations on the impact of food formulation on the food bolus and its characteristics, for example, in the design of new foods for targeted populations (the elderly, children, non-cooperative human subjects, and even animals). It can also be used to examine the oral release of nutrients and of active, toxic substances and food contaminants from the matrix being chewed, for example, to evaluate the role of the oral cavity in the balance between the oral bioaccessibility and bioavailability of nutrients. Using a mastication simulator is also very useful for the production of specific food boluses to address the fate of the food in the digestive tract. Numerous studies are conducted with a simulated bolus produced by mincing, mixing or crushing a food sample without any control of particle size distribution and saliva action in the pre-swallow food bolus before its subjection to artificial digestion (Bornhorst &

101 Singh, 2013; Mandalari et al., 2018; Minekus et al., 2014). A food bolus produced by a
102 mastication simulator is more realistic, closer to the material passing through the esophagus
103 and arriving in the stomach, and it can be subjected to artificial, static or dynamic digestion
104 more pertinently. Indeed the oral step of food destructuration by mastication is very often
105 badly simulated in digestion studies and sometimes not considered at all, even when
106 remarkable *in vitro* digestion models are used (Dupont et al., 2018; Hoebler et al., 2002; Kong
107 & Singh, 2008b; Minekus et al., 2014; Shani-Levi et al., 2017). Since the AM^2 can produce a
108 realistic food bolus, its association with digestion equipment such as the DIDGI® apparatus
109 (Ménard et al., 2014; Sayd, Chambon, & Santé-Lhoutellier, 2016) makes it possible to assess
110 the role of mastication in the subsequent steps of digestion. From this perspective, studies
111 could be performed on the role of oral microbiota in bolus formation, nutrient release and
112 digestion when mixed with the intestinal microbiota.

113 As already reviewed, several artificial mastication simulators have been developed but
114 most of them are unable to produce a food bolus for further analysis or use (Morell,
115 Hernando, & Fiszman, 2014; Peyron & Woda, 2016). The Artificial Masticatory Advanced
116 Machine (AM^2) was developed and validated against *in vivo* data to address multiple issues
117 regarding in-mouth food processing and the resulting food bolus (Woda et al., 2010a). Its
118 purpose was to simulate the result of mastication, *i.e.* to produce a food bolus with properties
119 similar to those produced by natural mastication, taking into account food oral management as
120 described in the literature (Chen, 2009). The anatomical features of the teeth and mouth were
121 not duplicated. On the contrary, the basic characteristics of jaw movement and other oral
122 parameters at work during chewing were mimicked so that the production of a pre-swallow
123 food bolus with the AM^2 obeys natural physiological laws (Lund & Kolta, 2006; Woda,
124 Foster, Mishellany, & Peyron, 2006). Consequently, after correctly selecting the
125 programming, the granulometry of the food boluses obtained at different times during the

126 chewing process were similar *in vitro* and *in vivo,* thus providing the basis for validating the
127 apparatus (Mishellany-Dutour et al., 2011; Woda et al., 2010a). Until now, very few of the
128 results obtained with this tool have been published (Peyron, Santé-Lhoutellier, François, &
129 Hennequin, 2018). This article presents an overview of several types of original results
130 obtained with the AM^2 in the fields of food and nutrition science related to oral processing.
131 The results presented in Fig.12 are the only ones adapted from the paper published previously
132 (Peyron et al., 2018). Other versions of the AM^2 masticator apparatus could be produced to
133 demand. Designing modified prototypes is also undoubtedly a realistic goal in view to
134 satisfaying specific needs to answer various potential scientific questions.
135

136 **2. The masticator apparatus AM^2**

137

138 *2.1 Description of the masticatory apparatus AM^2*

139

140 The complete description of the apparatus and its validation were provided in previous
141 papers (Mishellany-Dutour et al., 2011; Woda et al., 2010a). Briefly, this tool is composed of
142 a cylindrical cavity that constitutes a masticatory chamber (Fig. 1). The two ends of the
143 cylindrical chamber are formed by a fixed masticatory disk (=''maxillary disk'') on one side,
144 and a mobile masticatory disk (=''mandibular disk'') on the other, to simulate the dental arch.
145 These masticatory disks have surfaces whose shape differs from human dental anatomy but
146 are similar to the contact surface area at work in human mastication. They are designed to
147 produce the same types of shear and compression stresses as those exerted *in vivo*. The mobile
148 masticatory disk is moved like a piston by two motors imparting translational and rotational
149 movements like those described during food management in the mouth during mastication. A
150 spring is located behind the fixed maxillary disk. The force generated for food compression

during mastication depends on the stiffness of the spring which is chosen as a function of food resistance (Mishellany-Dutour et al., 2011; Woda et al., 2010a). The actions of shearing food material, as well as gathering particles and transporting them to the masticatory surface are performed by the rotational movement of the mandibular disk, as the tongue would do. Water or a validated artificial saliva (Roger-Leroi, Mishellany-Dutour, Woda, Marchand, & Peyron, 2012) can be injected at the beginning and / or by staggered injections during the masticatory sequence.

2.2 Programming of the masticatory apparatus AM^2

Whatever the objective of the study, a common procedure is used for all foods when programming the AM^2 masticatory apparatus. It is always based on preliminary *in vivo* data collected both from subjects presenting the oral conditions selected for the experiment (healthy oral condition, edentate, etc.) and from the granulometric characteristics of the food bolus collected (Jalabert-Malbos et al., 2007; Ngom, Diagne, Aïdara-Tamba, & Sene, 2007; van der Bilt, Abbink, Mowlana, & Heath, 1993). The cumulative particle size distribution curves obtained are used for comparing *in vivo* and *in vitro* food boluses. The median particle size d50, extracted from the cumulative curve by extrapolation and projection on the x-axis, and defined as the theoretical sieve size through which 50% of the bolus mass can pass, is also used for comparisons. To obtain the most efficient programming, comparisons are made after successive adjustments of the AM^2 parameters until the *in vivo* and *in vitro* curves and d50 values match each other.

The adjustments made to the mechanical parameters can be of many types and depend on the food masticated.. Briefly, the range of forces to be applied will depend on the spring chosen according its stiffness (from 10.38 to 35.2 N/mm). As examples derived from

experimental tests, the mastication of a French baguette sample would be done with the 10.38 N/mm spring and a raw carrot sample with the 25 N/mm spring. The number of masticatory cycles is always obtained from the in vivo measurements of mastication of the food considerd. A large angle (around 200°) is chosen for a "fibrous" food, such as meat needing more shear stress to be disrupted compared to a brittle food such as cookies for which an angle of 90° is enough. Various rotational angles are also tested for gathering particles. The temperature of the masticatory chamber can be regulated at 36°C if the oral processing studied is temperature dependent. Water, human or artificial saliva can be used for mastication. The flow and composition of artificial saliva with respect to mucin and enzyme contents (amylase and lipase, for example) can be chosen according to the scientific issue to be addressed (Roger-Leroi et al., 2012). Fig. 2 shows an example of the programming approach in which two numbers of masticatory cycles and two levels of spring-induced forces were sequentially changed in the programming of the masticator apparatus to find the best fit with the *in vivo* particle size distribution curve in meat bolus analyzed at swallowing time. As shown in Fig. 2, by performing the same number of masticatory cycles as observed *in vivo*, the stiff spring (23.5 N/mm) overly-disrupted the meat bolus compared to the softer spring (18.2 N/mm). Finally, after progressive adjustment, the mean particle size distribution curves of the meat boluses obtained *in vivo* and *in vitro* in the conditions selected were identical at swallowing time (Fig. 2).

Similar *in vivo* - *in vitro* agreements have also been reported for pork meat and green olive (Peyron & Woda, 2016). Several examples are shown in Fig. 3 for gherkins, a mouthful of beef, minced beef, Frankfurt sausages, coconut and a pasta product. *In vivo* - *in vitro* agreements were also observed when mastication was interrupted at different times while chewing carrots or peanuts (Mishellany-Dutour et al., 2011a) and with pork meat in Fig. 4 (A and B). Fig. 4c shows that the difference observed in the particle size distributions, as well as

in d50 values, between two types of bread *in vivo* can be completely reproduced *in vitro.*

A complete validation of the apparatus should also be performed by considering other physical properties of the food bolus, for example, using an instrumental Texture Profile Analysis (TPA) test consisting in applying double compression on the food bolus. Despite its empirical nature, this test is useful to compare the properties of food boluses collected at different times of the masticatory sequence, for different food matrixes or in different oral conditions, both for *in vivo* or *in vitro* food boluses (Peyron et al., 2011). Hardness, recoverability, adhesiveness and springiness values can be extracted from each TPA curve for bolus mechanical characterization. As an example of this interesting use, Fig. 5A illustrates that a piece of French bread (baguette) is softened, rendered less elastic and less cohesive as the *in vivo* masticatory sequence progresses. Physical characteristics obtained for in vivo and in vitro boluses can be compared to attest the effectiveness of the masticator apparatus (Fig. 5B). The possible addition of water, or human or artificial saliva in the AM^2 is undoubtedely a key parameter in preparing a food bolus for instrumental measurements since it provides the specific rheological environment of the food after mastication. A comparison of the physical characteristics of the *in vitro* food boluses obtained with these different fluids would be informative regarding the role of the specific components such as enzymes and proteins. Nevertheless, great care must be taken in the treatment of TPA test variables which are not absolute values, they are often misinterpreted, and they can only be used in a comparative way (Nishinari & Fang, 2018).

All the *in vivo* data used to validate or to program the masticator apparatus were obtained after collecting the informed consent of each subject and experiments were performed in accordance with different local and national ethical agreements (CE-CIC-GREN-10/06-#5044; DREAM-2013-A00096-39).

226 **3. Substance release in the mouth**

227

228 Chewing a solid matrix causes the release of numerous substances within the mouth,
229 normally resulting in a satisfactory sensory experience from food containing juice or tasty
230 molecules, for example. It can also favor nutrient extraction as early as the oral step.
231 Conversely, such release must be avoided when the foods or the non-nutritive products
232 contain toxic or pollutant substances as can happen, for example, when a child is chewing or
233 gnawing something containing a non-edible or toxic substance. However, obtaining the
234 delivery of an active substance could be the objective pursued, for example, with medicated
235 chewing-gums (Konar, Palabiyik, Toker, & Sagdic, 2016).

236 An artificial device is mandatory when the precise concentration of a compound released
237 during mastication has to be determined. Fig. 6A shows the release curve along the
238 mastication of a non-alimentary product containing two different concentrations of a toxic
239 substance. The kinetics and level of release could be determined and used for toxicity risk
240 analysis. This approach is obviously applicable for quantifying the release of toxic or
241 contaminant compounds from any alimentary product.

242 Most often the substances analyzed released from food or from any non-nutritive product
243 during chewing are dissolved or dispersed in an oral liquid composed of a mix of saliva and
244 liquids expressed in the food matrix. Determining the true oral concentration of the substance
245 of interest implies the separation of these two types of liquids. In usual conditions, the volume
246 of saliva cannot be determined because the saliva flow undergoes a large number of
247 unpredictable variations and due to frequent intermediate swallows (Hiiemae & Palmer,
248 1999). AM^2 can be used to distinguish between saliva and the juice extracted from food.

249 In the examples shown in Fig. 6 (B and C), mango juice was collected *in vivo* after
250 chewing with the fruit placed within an impermeable plastic bag (Fig. 6B) and *in vitro* with

251 the fruit in the AM^2 (Fig. 6C). The weight of the mango juice collected *in vitro* after 24
252 masticatory cycles was similar to the amount obtained after *in vivo* chewing performed with
253 24 cycles. With this AM^2 based design, the juice released from the matrix disruption was
254 estimated to be about 20% of the initial weight of the mango sample (Fig. 6Bb). Without the
255 bag, the *in vivo* mastication produced a liquid phase composed of juice and saliva,
256 representing about 40% of the bolus weight. As expected, the amount of mango juice
257 extracted also depended on the number of chewing cycles (Fig. 6C).

258 Fig. 7 shows the kinetics of glucose release in saliva during *in vivo* and *in vitro* mastication
259 of a chewing gum. In this experiment, the gum was weighed before and after 1, 3, 6, 10 and
260 12 min chewing. The gum weight was expressed as the cumulative course of weight loss
261 observed along the masticatory sequences (Fig. 7A). The glucose was determined in saliva
262 samples at the same time points of the masticatory sequence (Fig. 7B). It was obtained with a
263 glucometer using the electrochemical determination of glucose oxidase activity compared to
264 the pre-established standard curve for glucose in saliva. The results were expressed as the
265 cumulative time course of the amount of glucose measured in saliva at the different time
266 points of the masticatory sequences. Mastication occurred either *in vivo* with 8 food bolus
267 samples obtained from five volunteers or *in vitro* with 8 repetitions performed with the
268 masticator apparatus. The greater proportion of glucose release occurred during the three first
269 minutes of mastication and the gum weight decreased mostly at the same time. Weight loss
270 and glucose release changes had similar time courses for *in vivo* and *in vitro* gum chewing.

271 This kind of experiment can be conducted, for example, for the development of a
272 medicated chewing gum designed to provide a slow steady release of the medicine contained
273 in it, favoring the release of a substance in saliva and its progressive absorption. A relevant
274 time-curve would be obtained with the AM^2 apparatus.

275

4. Oral bioaccessibility of nutrients, digestion studies

The role of mastication and considerable food matrix disruption in the absorption of nutrients is important (Pennings et al., 2013; Rémond et al., 2007). Sieving through a tissue mesh enables the separate collection of liquid and solid phases of the food bolus, giving access to the role of oral processes in nutrient bioaccessibility and digestion. This method has been successfully applied in *in vitro* food boluses. Fig. 8 (ABC) shows free-iron release from ~~the~~ a meat matrix during the mastication of either a beef sample or its minced counterpart. Although the bolus of minced meat was normally chewed in fewer masticatory cycles than the same meat not minced (10 *versus* 32 cycles), it produced a bolus composed of more small particles (Fig. 8A). Free-iron release from the matrix (Fig. 8B and C) rose significantly in both saliva and bolus material when the meat had been minced before mastication (12 replicates for iron determination). Fig. 8D shows that the amount of β-carotenes released in saliva during the *in vivo* mastication of raw carrot increased along the masticatory process, obviously due to matrix disruption. This kind of measurement is totally feasible *in vitro* with the masticatory simulator. The analysis of the bioaccessibility of carotenoids could undoubtedly be performed in vitro with the AM^2, since the release of nutrients from plant foods is largely dependent on food breakage during mastication and could be decisive for their bioavailability (Faulks & Southon, 2005; Hedrén et al., 2002; Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Oral bioaccessibility is also a relevant issue for other macro and micronutrients such as in the case of lipids in almonds (Cassady, Hollis, Fulford, Considine, & Mattes, 2009; Ellis et al., 2004; Grundy et al., 2015).

Fig. 9 illustrates the first step of digestion occurring in the mouth during mastication and quantified by the production of maltose after the oral hydrolysis of starch by salivary amylase. Pieces of different breads (2.5 g) were introduced in the AM^2. Mastication was carried out (20

301 masticatory cycles) either with water (Volvic®) or human saliva (1.6 mL as estimated by
302 weighting *in vivo* food boluses collected during the programming of the AM^2 apparatus).
303 Logically, as expected, the concentration of maltose was higher with human saliva than with
304 water, but also differed regarding the type of bread chewed. The mastication of pieces of
305 white bread led to significantly higher hydrolysis of maltose than the mastication of whole
306 wheat bread of the same weight. This observation confirmed the initiatory role of mastication
307 in digestion by disintegrating food into smaller particles that are impregnated by saliva
308 (Hoebler et al., 1998; Pentikäinen et al., 2019; Tournier, Grass, Zope, Salles, & Bertrand,
309 2012).

310 Although the major mechanical reduction of food occurs during mastication, it is
311 completed in the stomach (Kong & Singh, 2011). This continuum of activity is exemplified in
312 Fig. 10a, which shows the granulometric characteristics measured first in the *in vitro* food
313 bolus of pasta obtained with the AM^2 just before swallowing, and then in the gastric
314 compartment after *in vitro* digestion. Moreover, this example also highlighted that the
315 stomach cannot totally overcome ~~the~~ failure in food reduction during deficient mastication
316 resulting in a bolus with numerous large particles. This must be taken into account,
317 considering that the level of disintegration of a food in the stomach depends on the structure
318 of the matrix (Kong & Singh, 2008a) and impacts gastric emptying (Pera et al., 2002), thus
319 assigning a specific role to mastication and the quality of the food bolus arriving in the
320 stomach. Large particles have also been shown to lengthen gastric emptying and to produce
321 lower glycemic and insulin blood responses than smaller particles (Ranawana, Clegg, Shafat,
322 & Henry, 2011). This could be an acceptable explanation for the lengthening of the whole
323 digestive process and the delay in nutrient postprandial appearance observed in edentulous
324 people (Rémond et al., 2007).

325 Fig. 10B shows the kinetics of protein concentration measured *in vitro* in the stomach

326 simulating digestion of an elderly person (in terms of pH kinetics and pepsin amount) after
327 the oral ingestion of meat. Using the masticator apparatus AM^2 with different programmings
328 to simulate either *in vitro* normal or deficient mastication, combined with an instrumental
329 gastric system, it appeared that large food fragments constituting the food bolus after deficient
330 *in vitro* mastication resulted in a significant deficit in protein release in elderly digestive
331 conditions.

332

333 **5. Biochemical modifications during oral processing**

334

335 The mastication simulator offers the possibility of exploring the chemical role of saliva and
336 modification of the food compounds occurring within the mouth while the food is being
337 transformed into a bolus ready to be swallowed. The various roles of saliva can be explored
338 since it can be added or not in the apparatus for *in vitro* mastication. For example, human
339 saliva is assumed to protect food compounds against oxidation (Moore, Calder, Miller, &
340 Rice-Evans, 1994). Thus the AM^2 allows testing the role of saliva by using *in vitro* no-saliva,
341 human saliva or any composition of artificial saliva containing enzymes or not, for example.
342 Fig. 11 (A and B) shows the effect of chewing on the oxidation of beef meat when the
343 artificial mouth was devoid of saliva. Lipid and protein oxidation in the food bolus was
344 assessed by changes in TBARS (ThioBarbituric Acid Reactive Substances) and by the
345 quantification of carbonyl groups (aldehydes and ketones), respectively. Three meat samples
346 were measured before chewing (raw meat, cooked meat, cooked + minced meat) and 12
347 boluses were obtained after *in vitro* mastication (just before swallowing). Without saliva,
348 significantly higher lipid oxidation was observed when the meat sample was chewed in
349 minced format rather than as a non-minced meat (Fig. 11A). The oxidation of proteins
350 occurring during chewing without saliva was also significant although less marked than for

351 lipids (Fig. 11B). These results confirm the potential occurrence of lipid and protein oxidation
352 and underline the essential antioxidant role of saliva in protecting food compounds from
353 oxidation (Qu et al., 2016). The more a food is chewed the more likely it will be impregnated
354 by saliva and the nutrients protected against oxidation. This aspect should be thoroughly
355 studied for various foods since it may have serious consequences for certain nutritional issues
356 as nutrient oxidation has known consequences on intestinal absorption (Bax et al., 2012).
357

358 **6. Effect of deficient mastication on food bolus processing**

359

360 The AM^2 can also simulate different dental states related to oral diseases, healthy
361 childhood and ageing conditions. Fig. 12 indicates the effects of different modalities of
362 impairment on the granulometry of poultry meatball boluses (Peyron et al., 2018). The *in vivo*
363 boluses were collected from seven subjects at swallowing threshold after normal mastication
364 (NM) composed of 17 masticatory cycles. As usual, preliminary *in vivo* experiments allowed
365 determining the main settings for *in vitro* mastication, and the simulation of deficient
366 mastication was performed with modifications of certain parameters. This introduced specific
367 deteriorations of the masticatory sequence in terms of the force and motility of oral elements,
368 as illustrated in Figure 2 for the choice of spring-induced forces. Two levels were set for
369 deficient force by using the spring of lowest stiffness together with an increasing offset
370 between the jaws at closing (Def Force 1 and Def Force 2) and deficient motility simulated by
371 two different low values of rotation angle (compared to normal programming) applied during
372 jaw confrontation (Def Motil 1 and Def Motil 2). A considerable decline in masticatory force
373 (Def Force 2 in Fig. 12) significantly changed the particle size distribution in the meatball
374 boluses towards a greater proportion of large particles ($p<0.001$). Motility deficiency also
375 significantly limited food fragmentation, with an impact corresponding to the level of the

deficiency ($p<0.001$). This difference was even more dramatic when the masticatory force and motility deficiencies (Def Motil & Force) were added together during mastication compared to other conditions ($p<0.001$). The description of the impact of gradual and cumulative deficiencies on the physical properties of boluses obtained with poultry meatballs has been described elsewhere (Peyron et al., 2018) and the results agreed with those obtained *in vivo* (Mishellany-Dutour, Renaud, Peyron, Rimek, & Woda, 2008; Woda et al., 2010b).

7. Measurement of forces applied during mastication

The AM^2 can measure forces developed during mastication with a strength sensor (Fig. 1). The specific approach described in Figs. 13 and 14 is mandatory. The large artifacts recorded from the force sensor included in the AM^2 came from frictions induced by the mobile jaw's forward and backward movements. This implied stopping all the movements for one or two seconds during which the force was measured. Fig. 13A shows the method used to calibrate the force sensor to a null force with an empty mastication chamber to overcome the artifacts. Fig. 13B shows an experiment on force measurements performed with gelatin samples as models (10 mm high, 20 mm diameter) presenting three levels of hardness but the same recoverability—(Peyron, Lassauzay, & Woda, 2002). The forces recorded during five successive cycles differed according to the level of hardness of the food models.

Fig. 14A shows an experiment where the forces ~~used~~ during a long sequence of 36 cycles were recorded during the chewing sequence of a banana sample (10 mm high and 20 mm diameter). During this experiment the moving jaw compressed the sample, while the fixed jaw moved back due to spring compression, leading to a partial deformation of the sample. The masticatory forces progressively decreased from 25.42± 0.81 N–at the first bite to near zero N at the last bite (5 replicates). A comparable force value (26.99 ± 1.08 N) was measured

with the Instron machine (Fig. 14B) performing a single compression test at 65% deformation (the same deformation range applied on the banana sample by the AM^2) on two banana samples taken from the same bunch as the sample used for the experiment presented in Fig. 14A. The resistance force obtained with the Instron machine when the sample was compressed (26.99 ± 1.08N) was comparable to that obtained with the AM^2 at the first bite. In another experiment presented in Fig. 14 (C and D), the AM^2 was used to mimick testing machine mode to test the effect of fruit ripening. The ripe and firm banana samples were subjected to five successive compressions. The resistance was higher (7.93 ± 1.22N) for the firm banana than for the ripe one (4.28 ± 1.04N).

8. Conclusion

For various reasons ranging from financial to ethical considerations, but also because it is not possible to access the interior of the mouth during mastication, the AM^2 masticator apparatus offers a unique approach in numerous fields of research in food science, including food design, oral health and nutrition issues. It facilitates experiments in particular when large samples of subjects or high numbers of replicates would be required *in vivo*. Also, the number of repetitions can be much smaller because the variations between them are due only to mechanical hazards that are much easier to control than biological intersubject variations. Finally, no ethical requirements are needed with *in vitro* experiments.

The AM^2 has certain limits. Contrary to many other articial masticators (Salles et al., 2007; van Ruth & Buhr, 2004), it does not currently offer the possibility of collecting volatile aromatic compounds during food disruption within the masticatory chamber. This limit, however, could be overcome technically if needed. At present, the choice of masticatory programming is based on several *in vivo* recordings followed by the adjustment of the AM^2

parameters. In the future, it may be possible to introduce a retrocontrol based on neural networks in the AM^2 which would allow adaptation to ongoing food bolus transformation, as is the case in *in vivo* mastication

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References

Bax, M.-L., Aubry, L., Ferreira, C., Daudin, J.-D., Gatellier, P., Rémond, D., & Santé-Lhoutellier, V. (2012). Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry*, *60*(10), 2569–2576. https://doi.org/10.1021/jf205280y

Bonnet, G., Batisse, C., Peyron, M.-A., Nicolas, E., & Hennequin, M. (2018). Which variables should be controlled when measuring the granulometry of a chewed bolus? A systematic review. *Journal of Texture Studies*. https://doi.org/10.1111/jtxs.12376

Bornhorst, G. M., & Singh, R. P. (2013). Kinetics of in Vitro Bread Bolus Digestion with Varying Oral and Gastric Digestion Parameters. *Food Biophysics*, *8*(1), 50–59. https://doi.org/10.1007/s11483-013-9283-6

Cassady, B. A., Hollis, J. H., Fulford, A. D., Considine, R. V., & Mattes, R. D. (2009). Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. *The American Journal of Clinical Nutrition*, *89*(3), 794–800. https://doi.org/10.3945/ajcn.2008.26669

Chen, J. (2009). Food oral processing—A review. *Food Hydrocolloids*, *23*(1), 1–25. https://doi.org/10.1016/j.foodhyd.2007.11.013

Chen, J. (2015). Food oral processing: Mechanisms and implications of food oral destruction. *Trends in Food Science & Technology*, *45*(2), 222–228. https://doi.org/10.1016/j.tifs.2015.06.012

Dupont, D., Alric, M., Blanquet-Diot, S., Bornhorst, G., Cueva, C., Deglaire, A., … Van den Abbeele, P. (2018). Can dynamic *in vitro* digestion systems mimic the physiological reality? *Critical Reviews in Food Science and Nutrition*, 1–17. https://doi.org/10.1080/10408398.2017.1421900

Ellis, P. R., Kendall, C. W. C., Ren, Y., Parker, C., Pacy, J. F., Waldron, K. W., & Jenkins, D. J. A. (2004). Role of cell walls in the bioaccessibility of lipids in almond seeds. *The American Journal of Clinical Nutrition*, *80*(3), 604–613. https://doi.org/10.1093/ajcn/80.3.604

Engelen, L., Fontijn-Tekamp, A., & Bilt, A. van der. (2005). The influence of product and oral characteristics on swallowing. *Archives of Oral Biology*, *50*(8), 739–746. https://doi.org/10.1016/j.archoralbio.2005.01.004

Faulks, R. M., & Southon, S. (2005). Challenges to understanding and measuring carotenoid bioavailability. *Biochimica Et Biophysica Acta*, *1740*(2), 95–100. https://doi.org/10.1016/j.bbadis.2004.11.012

Funami, T. (2016). The Formulation Design of Elderly Special Diets: Formulation design for elderly. *Journal of Texture Studies*, *47*(4), 313–322. https://doi.org/10.1111/jtxs.12202

Gao, J., Wong, J. X., Lim, J. C.-S., Henry, J., & Zhou, W. (2015). Influence of bread structure on human oral processing. *Journal of Food Engineering*, *167*, 147–155. https://doi.org/10.1016/j.jfoodeng.2015.07.022

Grundy, M. M. L., Grassby, T., Mandalari, G., Waldron, K. W., Butterworth, P. J., Berry, S. E. E., & Ellis, P. R. (2015). Effect of mastication on lipid bioaccessibility of almonds in a randomized human study and its implications for digestion kinetics, metabolizable energy, and postprandial lipemia. *The American Journal of Clinical Nutrition*, *101*(1), 25–33. https://doi.org/10.3945/ajcn.114.088328

Hedrén, E., Diaz, V., & Svanberg, U. (2002). Estimation of carotenoid accessibility from carrots determined by an in vitro digestion method. *European Journal of Clinical Nutrition*, *56*(5), 425–430. https://doi.org/10.1038/sj.ejcn.1601329

Hiiemae, K., Heath, M. R., Heath, G., Kazazoglu, E., Murray, J., Sapper, D., & Hamblett, K. (1996). Natural bites, food consistency and feeding behaviour in man. *Archives of Oral Biology*, *41*(2), 175–189.

Hiiemae, K. M., & Palmer, J. B. (1999). Food transport and bolus formation during complete feeding sequences on foods of different initial consistency. *Dysphagia*, *14*(1), 31–42. https://doi.org/10.1007/PL00009582

Hoebler, C., Devaux, M. F., Karinthi, A., Belleville, C., & Barry, J. L. (2000). Particle size of solid food after human mastication and in vitro simulation of oral breakdown. *International Journal of Food Sciences and Nutrition*, *51*(5), 353–366.

Hoebler, C., Karinthi, A., Devaux, M.-F., Guillon, F., Gallant, D. J. G., Bouchet, B., … Barry, J.-L. (1998). Physical and chemical transformations of cereal food during oral digestion in human subjects. *British Journal of Nutrition*, *80*(05), 429. https://doi.org/10.1017/S0007114598001494

Hoebler, C., Lecannu, G., Belleville, C., Devaux, M.-F., Popineau, Y., & Barry, J.-L. (2002). Development of an in vitro system simulating bucco-gastric digestion to assess the physical and chemical changes of food. *International Journal of Food Sciences and Nutrition*, *53*(5), 389–402. https://doi.org/10.1080/0963748021000044732

Hwang, J., Kim, D.-K., Bae, J. H., Kang, S. H., Seo, K. M., Kim, B. K., & Lee, S. Y. (2012). The effect of rheological properties of foods on bolus characteristics after mastication. *Annals of Rehabilitation Medicine*, *36*(6), 776–784. https://doi.org/10.5535/arm.2012.36.6.776

Jalabert-Malbos, M.-L., Mishellany-Dutour, A., Woda, A., & Peyron, M.-A. (2007). Particle size distribution in the food bolus after mastication of natural foods. *Food Quality and Preference*, *18*(5), 803–812. https://doi.org/10.1016/j.foodqual.2007.01.010

Koç, H., Çakir, E., Vinyard, C. J., Essick, G., Daubert, C. R., Drake, M. A., … Foegeding, E. A. (2014). Adaptation of Oral Processing to the Fracture Properties of Soft Solids: Oral Processing and Fracture Properties. *Journal of Texture Studies*, *45*(1), 47–61. https://doi.org/10.1111/jtxs.12051

Konar, N., Palabiyik, I., Toker, O. S., & Sagdic, O. (2016). Chewing gum: Production, quality parameters and opportunities for delivering bioactive compounds. *Trends in Food Science & Technology*, *55*, 29–38. https://doi.org/10.1016/j.tifs.2016.07.003

Kong, F., & Singh, R. P. (2008). A model stomach system to investigate disintegration kinetics of solid foods during gastric digestion. *Journal of Food Science*, *73*(5), E202-210. https://doi.org/10.1111/j.1750-3841.2008.00745.x

Kong, Fanbin, & Singh, R. P. (2011). Solid Loss of Carrots During Simulated Gastric Digestion. *Food Biophysics*, *6*(1), 84–93. https://doi.org/10.1007/s11483-010-9178-8

Lund, J. P., & Kolta, A. (2006). Generation of the central masticatory pattern and its modification by sensory feedback. *Dysphagia*, *21*(3), 167–174. https://doi.org/10.1007/s00455-006-9027-6

Mandalari, G., Parker, M. L., Grundy, M. M.-L., Grassby, T., Smeriglio, A., Bisignano, C., … Wilde, P. J. (2018). Understanding the Effect of Particle Size and Processing on Almond Lipid Bioaccessibility through Microstructural Analysis: From Mastication to Faecal Collection. *Nutrients*, *10*(2). https://doi.org/10.3390/nu10020213

Ménard, O., Cattenoz, T., Guillemin, H., Souchon, I., Deglaire, A., Dupont, D., & Picque, D. (2014). Validation of a new in vitro dynamic system to simulate infant digestion. *Food Chemistry*, *145*, 1039–1045. https://doi.org/10.1016/j.foodchem.2013.09.036

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Mishellany-Dutour, A., Peyron, M.-A., Croze, J., François, O., Hartmann, C., Alric, M., & Woda, A. (2011a). Comparison of food boluses prepared in vivo and by the AM2 mastication simulator. *Food Quality and Preference*, *22*(4), 326–331. https://doi.org/10.1016/j.foodqual.2010.12.003

Mishellany-Dutour, A., Peyron, M.-A., Croze, J., François, O., Hartmann, C., Alric, M., & Woda, A. (2011b). Comparison of food boluses prepared in vivo and by the AM2 mastication simulator. *Food Quality and Preference*, *22*(4), 326–331. https://doi.org/10.1016/j.foodqual.2010.12.003

Mishellany-Dutour, A., Renaud, J., Peyron, M.-A., Rimek, F., & Woda, A. (2008). Is the goal of mastication reached in young dentates, aged dentates and aged denture wearers? *The*

British Journal of Nutrition, *99*(1), 121–128. https://doi.org/10.1017/S0007114507795284

Moore, S., Calder, K. A., Miller, N. J., & Rice-Evans, C. A. (1994). Antioxidant activity of saliva and periodontal disease. *Free Radical Research*, *21*(6), 417–425.

Morell, P., Hernando, I., & Fiszman, S. M. (2014). Understanding the relevance of in-mouth food processing. A review of in vitro techniques. *Trends in Food Science & Technology*, *35*(1), 18–31. https://doi.org/10.1016/j.tifs.2013.10.005

Ng, G. C. F., Gray-Stuart, E. M., Morgenstern, M. P., Jones, J. R., Grigg, N. P., & Bronlund, J. E. (2017). The slip extrusion test: A novel method to characterise bolus properties. *Journal of Texture Studies*, *48*(4), 294–301. https://doi.org/10.1111/jtxs.12254

Ngom, P. I., Diagne, F., Aïdara-Tamba, A. W., & Sene, A. (2007). Relationship between orthodontic anomalies and masticatory function in adults. *American Journal of Orthodontics and Dentofacial Orthopedics*, *131*(2), 216–222. https://doi.org/10.1016/j.ajodo.2005.03.027

Nishinari, K., & Fang, Y. (2018). Perception and measurement of food texture: Solid foods. *Journal of Texture Studies*, *49*(2), 160–201. https://doi.org/10.1111/jtxs.12327

Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science*, *76*(1), R6–R15. https://doi.org/10.1111/j.1750-3841.2010.01957.x

Pennings, B., Groen, B. B. L., van Dijk, J.-W., de Lange, A., Kiskini, A., Kuklinski, M., … van Loon, L. J. C. (2013). Minced beef is more rapidly digested and absorbed than beef steak, resulting in greater postprandial protein retention in older men. *The American Journal of Clinical Nutrition*, *98*(1), 121–128. https://doi.org/10.3945/ajcn.112.051201

571 Pentikäinen, S., Koistinen, V., Kolehmainen, M., Poutanen, K., Hanhineva, K., & Aura, A.-
572 M. (2019). Mastication-induced release of compounds from rye and wheat breads to
573 saliva. *Food Chemistry*, *270*, 502–508. https://doi.org/10.1016/j.foodchem.2018.07.110
574 Pera, P., Bucca, C., Borro, P., Bernocco, C., De, L. A., & Carossa, S. (2002). Influence of
575 mastication on gastric emptying. *Journal of Dental Research*, *81*(3), 179–181.
576 Peyron, M. A., Lassauzay, C., & Woda, A. (2002). Effects of increased hardness on jaw
577 movement and muscle activity during chewing of visco-elastic model foods.
578 *Experimental Brain Research*, *142*(1), 41–51. https://doi.org/10.1007/s00221-001-0916-
579 5
580 Peyron, M.-A., Gierczynski, I., Hartmann, C., Loret, C., Dardevet, D., Martin, N., & Woda,
581 A. (2011). Role of physical bolus properties as sensory inputs in the trigger of
582 swallowing. *PloS One*, *6*(6), e21167. https://doi.org/10.1371/journal.pone.0021167
583 Peyron, M.-A., Santé-Lhoutellier, V., François, O., & Hennequin, M. (2018). Oral declines
584 and mastication deficiencies cause alteration of food bolus properties. *Food & Function*,
585 *9*(2), 1112–1122. https://doi.org/10.1039/c7fo01628j
586 Peyron, Marie-Agnès, & Woda, A. (2016). An update about artificial mastication. *Current*
587 *Opinion in Food Science*, *9*, 21–28. https://doi.org/10.1016/j.cofs.2016.03.006
588 Qu, X. M., Wu, Z. F., Pang, B. X., Jin, L. Y., Qin, L. Z., & Wang, S. L. (2016). From Nitrate
589 to Nitric Oxide: The Role of Salivary Glands and Oral Bacteria. *Journal of Dental*
590 *Research*, *95*(13), 1452–1456. https://doi.org/10.1177/0022034516673019
591 Ranawana, V., Clegg, M. E., Shafat, A., & Henry, C. J. (2011). Postmastication digestion
592 factors influence glycemic variability in humans. *Nutrition Research (New York, N.Y.)*,
593 *31*(6), 452–459. https://doi.org/10.1016/j.nutres.2011.05.006
594 Rémond, D., Machebeuf, M., Yven, C., Buffière, C., Mioche, L., Mosoni, L., & Patureau
595 Mirand, P. (2007). Postprandial whole-body protein metabolism after a meat meal is

influenced by chewing efficiency in elderly subjects. *The American Journal of Clinical Nutrition*, *85*(5), 1286–1292. https://doi.org/10.1093/ajcn/85.5.1286

Roger-Leroi, V., Mishellany-Dutour, A., Woda, A., Marchand, M., & Peyron, M. A. (2012). Substantiation of an artificial saliva formulated for use in a masticatory apparatus. *Odonto-Stomatologie Tropicale = Tropical Dental Journal*, *35*(138), 5–14.

Salles, C., Tarrega, A., Mielle, P., Maratray, J., Gorria, P., Liaboeuf, J., & Liodenot, J.-J. (2007). Development of a chewing simulator for food breakdown and the analysis of in vitro flavor compound release in a mouth environment. *Journal of Food Engineering*, *82*(2), 189–198. https://doi.org/10.1016/j.jfoodeng.2007.02.008

Sayd, T., Chambon, C., & Santé-Lhoutellier, V. (2016). Quantification of peptides released during in vitro digestion of cooked meat. *Food Chemistry*, *197 Pt B*, 1311–1323. https://doi.org/10.1016/j.foodchem.2015.11.020

Shani-Levi, C., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet-Diot, S., … Lesmes, U. (2017). Extending in vitro digestion models to specific human populations: Perspectives, practical tools and bio-relevant information. *Trends in Food Science & Technology*, *60*, 52–63. https://doi.org/10.1016/j.tifs.2016.10.017

Shiozawa, K., Kohyama, K., & Yanagisawa, K. (2003). Relationship between Physical Properties of a Food Bolus and Initiation of Swallowing. *Japanese Journal of Oral Biology*, *45*(2), 59–63. https://doi.org/10.2330/joralbiosci1965.45.59

Tournier, C., Grass, M., Zope, D., Salles, C., & Bertrand, D. (2012). Characterization of bread breakdown during mastication by image texture analysis. *Journal of Food Engineering*, *113*(4), 615–622. https://doi.org/10.1016/j.jfoodeng.2012.07.015

van der Bilt, A., Abbink, J. H., Mowlana, F., & Heath, M. R. (1993). A comparison between data analysis methods concerning particle size distributions obtained by mastication in

man. *Archives of Oral Biology*, *38*(2), 163–167. https://doi.org/10.1016/0003-9969(93)90202-W

van Ruth, S. M., & Buhr, K. (2004). Influence of mastication rate on dynamic flavour release analysed by combined model mouth/proton transfer reaction–mass spectrometry. *International Journal of Mass Spectrometry*, *239*(2–3), 187–192. https://doi.org/10.1016/j.ijms.2004.08.009

Woda, A., Foster, K., Mishellany, A., & Peyron, M. A. (2006). Adaptation of healthy mastication to factors pertaining to the individual or to the food. *Physiology & Behavior*, *89*(1), 28–35. https://doi.org/10.1016/j.physbeh.2006.02.013

Woda, A., Mishellany-Dutour, A., Batier, L., François, O., Meunier, J.-P., Reynaud, B., … Peyron, M.-A. (2010a). Development and validation of a mastication simulator. *Journal of Biomechanics*, *43*(9), 1667–1673. https://doi.org/10.1016/j.jbiomech.2010.03.002

Woda, A., Mishellany-Dutour, A., Batier, L., François, O., Meunier, J.-P., Reynaud, B., … Peyron, M.-A. (2010b). Development and validation of a mastication simulator. *Journal of Biomechanics*, *43*(9), 1667–1673. https://doi.org/10.1016/j.jbiomech.2010.03.002

Woda, A., Nicolas, E., Mishellany-Dutour, A., Hennequin, M., Mazille, M.-N., Veyrune, J.-L., & Peyron, M.-A. (2010). The masticatory normative indicator. *Journal of Dental Research*, *89*(3), 281–285. https://doi.org/10.1177/0022034509357022

Legends

Figure1: Schematic representation of the AM^2 artificial masticator with insert of an open view of the mastication chamber in which the fixed and mobile masticatory disks are shown. The whole apparatus is 130 cm long and 30 cm in height. The masticatory chamber (19.5 x 9.5 cm) is located in the continuation of the shaft holding the mobile masticatory disk.

Figure 2: Example of AM^2 programming for meat mastication. Mean particle size distribution curves are expressed as cumulative weight percentages of particles passing through each sieve during manual sieving. The force generated for food compression during mastication depends on the stiffness of the spring which must be chosen as a function of food resistance. The curve obtained with the spring 3 (18.2 N/mm of spring stiffness; n = 4) and 22 masticatory cycles gave the best fit with the mean *in vivo* curve (n = 8). Spring 3 with only 20 cycles gave a higher median value (= *d50*, theoretical sieve letting pass 50% particle mass), indicating a poorly prepared food bolus with larger particles (n = 4). Spring 4 (23.5 N/mm of spring stiffness) was harder and gave a lower *d50* value when used with 22 masticatory cycles, indicating excess ~~in~~ food bolus disruption (n = 4). For the sake of readability, standard deviations are not drawn.

Figure 3: Comparisons between mean particle size distribution curves of boluses obtained *in vivo* and *in vitro* from different food types. Piece of beef: cooked samples, 2cm square and 1cm thick. Minced beef: same cooked meat, molded in the same sample shape as non-minced meat. Frankfurt sausage: cylindrical samples of 6.5g. Pasta products: cooked Fusilli pasta in 7g bite. Coconut: 1cm square and 1cm thick sample. Gherkins: cylindrical sample of 3.5g. Each point represents the weight of the particles (in %) retained in the corresponding sieve size added to the sum of all the preceding points (cumulative curve). The similarity required between *in vivo* and *in vitro* bolus granulometry was not limited to the median particle size (*d50=50% on cumulative curve)* but sought for the whole mean particle size distribution curves. In these examples, better fitting could be obtained for minced meat and gherkins if further studies were carried out. For the sake of readability, standard deviations are not drawn.

Figure 4: Comparisons between mean particle size distribution curves for boluses obtained *in vivo* (A) and *in vitro* (B). The masticatory sequence of pork meat (2cm cubes) was interrupted

669 after one, two, three and four quarters of the *in vivo* (A) and *in vitro* (B) chewing sequences
670 (0.25, 0.5, 0.75 and 1; n=10). In C, particle size distributions were obtained for two types of
671 bread (white and whole wheat breads, sample = 1 /4 of a 2cm thick slice cut in a French
672 baguette) after *in vivo* and *in vitro* mastication (n=10). The difference observed *in vivo*
673 between the two types of bread can be completely reproduced *in vitro*. For the sake of
674 readability, standard deviations are not drawn.

675 Figure 5. A: Evolution of four physical characteristics of bread boluses produced *in vivo* from
676 a French baguette after one, two or three thirds of the complete chewing sequence (1/3, 2/3,
677 swallowing; n = 10 subjects x 2 repetitions; sample = 1 /0 of a 2cm thick slice). B:
678 Comparison of hardness and recoverability of pasta boluses produced *in vivo* and *in vitro* (n =
679 5 subjects or replicates; cooked Fusilli pasta in 7g bite). The food bolus was subjected to a
680 double compression test during a Texture Profile Analysis test (TPA) performed with an
681 Instron machine equipped with a flat piston head (Ø 28 mm) moved at a constant
682 displacement rate of 50 $mm.min^{-1}$, a cylindrical container (int. Ø 35 mm, ext. Ø 40 mm) and a
683 500 N load cell. The bolus was spread at the bottom of the cylindrical container and subjected
684 to 65% deformation of its initial height. In this example, hardness (N), adhesiveness (N.s),
685 elasticity and recoverability (both unitless) were extracted from the TPA curves (mean and
686 SD). Physical characteristics can be obtained in the same manner for *in vitro* boluses
687 produced with the masticator apparatus AM^2.

688 Figure 6. A: Curve of a contaminant released in saliva along the mastication of a non-
689 alimentary product. The two curves were obtained for the same product containing two
690 different concentrations of the same toxic substance. B: Liquid content in a bolus of fruit (in
691 % of bolus weight, mean and SD) after 24 *in vivo* masticatory cycles performed in normal
692 conditions and with the fruit sample placed in a bag while chewing to separate juice from

693 saliva (n=10; mean and SD). C: Percentage of juice extracted from fruit after 15 and 24 *in*
694 *vitro* masticatory cycles with AM^2 (n = 10, mean and SD).

695 Figure 7. Chewing a non-alimentary gum. Cumulative loss of gum weight (A; in %, mean and
696 SD) and cumulative concentration of glucose released in saliva (B; in g, mean and SD) were
697 obtained from different time point measurements along 12 minutes of in *vivo* and *in vitro*
698 mastication of the gum sample (n=8).

699 Figure 8. Impact of food structure on free-iron and carotene release in saliva. A: Bolus of
700 minced meat analyzed after normal mastication consisting of 10 cycles while the meat sample
701 (2x2x2 cm) needed 32 masticatory cycles to produce swallowable boluses (mean and SD). B
702 and C: At the end of mastication, free-iron release was greater for minced than for mouthful
703 meat, both in the solid part of the bolus and in saliva. D: Examples of the oral release of
704 carotene measured by HPLC in the salivary part of the bolus. For readability, spectra of beta-
705 carotenes determined in three saliva samples were superimposed according to the elution time
706 on the x-axis. It clearly shows an increase of beta-carotene release in saliva with the progress
707 of the masticatory sequence from the boluses collected at the beginning, in the middle of the
708 masticatory sequence, and when the bolus was ready to be swallowed.

709 Figure 9. Production of maltose in the mouth during the artificial mastication of whole wheat
710 and white bread samples, with the addition of water or saliva as the oral liquid (n=10; mean
711 and SD). All differences were significant.

712 Figure 10. A: Mean particle size distribution curve in the swallowable bolus after *in vitro*
713 mastication of pasta and in the gastric compartment after *in vitro* gastric digestion. B: Kinetics
714 of protein appearance in a simulated aged gastric compartment after normal and deficient
715 mastication simulated with the AM^2 (n = 3; mean and SD). Protein concentration was

716 significantly lower (*) from 90 min digestion in deficient mastication compared to normal
717 mastication.

718 Figure 11. Lipid and protein oxidation during *in vitro* mastication in the absence of saliva. A:
719 Lipid oxidation during *in vitro* mastication without saliva, measured by malondialdehyde
720 appearance (MDA) as thiobarbituric acid reactive substances (TBARS). B: Protein oxidation
721 during *in vitro* mastication without saliva measured by the quantity of carbonyl groups
722 (aldehydes and ketones present in the food bolus (reaction with dinitrophenylhydrazine
723 (DNPH).

724 Figure 12. Effects of two levels of deficient force (Def Force 1 & 2) and motility (Def Motil 1
725 & 2), and their combination, on the granulometry of poultry meatball boluses obtained with *in*
726 *vitro* mastication (n=10; mean and SD). *d50* value (in mm) is the median value of the particle
727 size distribution in the food bolus and defined as the theoretical sieve size through which 50%
728 of the mass can pass. Comparison was performed with normal mastication (NM). Significant
729 differences against NM d50 values were observed from Def Force 2 condition. *Data adapted*
730 *from Peyron et al, 2018*

731 Figure 13. Calibration (A) and measurement (B) of the resistance to compression of three
732 gelatine products (Ø 2 cm; height 1 cm) of different harnesses. Forces are measured with the
733 strength sensor along the shaft actuating the mobile masticatory disk. A: the AM^2 is activated
734 with a pure translation movement, with an empty chamber and for five successive cycles. At
735 the end of each cycle, the mobile (mandibular) jaw is stopped at initial contact, and contact
736 for two seconds, without applying forces. Forces recorded in the translational axis with the
737 strength sensor are in Newtons (N). The whole procedure lasted 23 seconds (s). The force
738 sensor records four types of events: ① friction of the mobile jaw against the mastication
739 chamber wall while the jaw is moving towards the useful range of movements; ② forces

resulting from the air compressed by the progress of the mobile jaw in the chamber; ③ forces resulting from negative air pressure while the jaw moves backwards; ④ null force while the jaw is stopped for 2 seconds with no pressure against the fixed jaw. B: Measuring the force during compression of three gelatines of different hardnesses. The events are the same as those described above except for event 4 which displays the forces in Newtons (N) applied during the mastication of the three gelatines differing in hardness. The translational movement was performed at 100 mm/s.

Figure 14. Forces recorded with the strength sensor of the AM^2 (Fig. 1) while chewing banana samples with 1.8 ml saliva, at 100 mm/s translational speed. A: Successive bites performed in 2 minutes chewing. The progressively decreasing force-from 25.42 ± 0.81N to almost zero, is highlighted by black circles and illustrates that the banana sample was progressively crushed and less resistant with the successive bites. B: Measures with an Instron machine performing a single compression test in replicates on 2 banana samples (2 cm diameter, 1 cm high) with a cylindrical piston head of 2.8 cm diameter. The values of forces in Newtons (26.99 ± 1.08 N) were similar to the value obtained during the first bite with AM^2 (25.42 ± 0.81 N). C and D: Measures of forces developed during a five-bite compression to test the effect of banana ripening on sample resistance with the AM^2 mimicking a testing machine mode (no saliva, spring of lowest stiffness, mobile disk actuated at 100mm/s).

accent europe

PROOFREADING CERTIFICATE

To whom it may concern,
the document bearing the title:

ADDRESSING VARIOUS CHALLENGES RELATED TO FOOD BOLUS AND NUTRITION WITH THE AM² MASTICATION SIMULATOR

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Canadian Journal of Civil Engineering
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Transport in Porous Media

Economics
Economic Modelling
International Journal of Public Sector Performance Labour
International Journal of Transport Economics

The environment
Aqua-Lac – UNESCO
Aquatic Toxicology
Chemosphere
Environmental Science and Pollution Research
Journal of Water Resource and Protection
Archives of environmental contaminants and toxicity
Earth Science Review
Ecotoxicology
Environment
Environment International
Forest Ecology and Management
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Microbial Ecology
Sciences of the Total Environment
Urban Water Journal
Water Sciences and technology Journal of Hazardous Materials
STOTEN
Vadose Zone Journal
Water Research

Food Science
International Journal of Food Science and Technology

Oceanography
Deep Sea Research
Journal of the Marine Biological Association of the United Kingdom

Structural mechanics, numerical simulation
ASME Journal of Applied Mechanics
ASME Journal of Computational Methods and Nonlinear Dynamics
ASME Journal of Vibration and Acoustics
Comptes Rendus de Mécanique
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Journal of Sound and Vibration
Mechanical Systems and Signal Processing
Journal of Vibration and Control
Materials Science and Engineering
Mechanical Systems and Signal Processing
Mechanism and Machine Theory
Nonlinear Dynamics
Proceedings of the ASME 2011 International Design Engineering Technical Conferences
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The Shock and Vibration Bulletin
Transactions of the ASME

Tribology
Journal of Tribology
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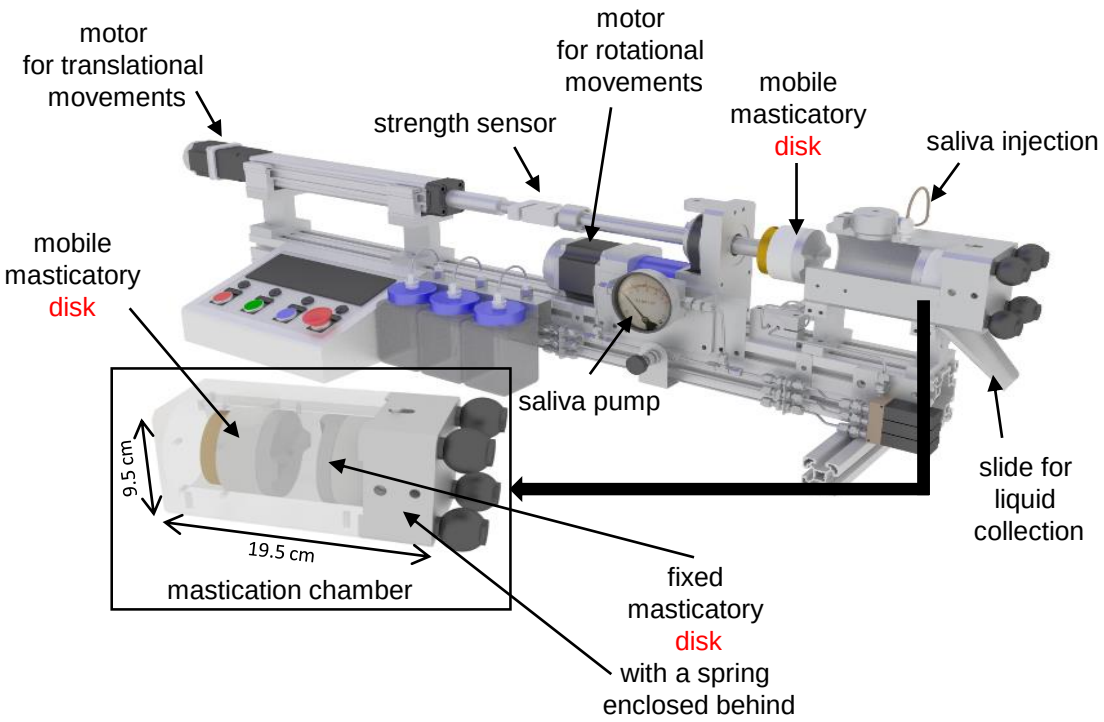

motor
for translational
movements
motor
for rotational
movements
mobile
masticatory
disk
saliva injection
strength sensor
mobile
masticatory
disk
saliva pump
9.5 cm
19.5 cm
mastication chamber
slide for
liquid
collection
fixed
masticatory
disk
with a spring
enclosed behind

Figure 1

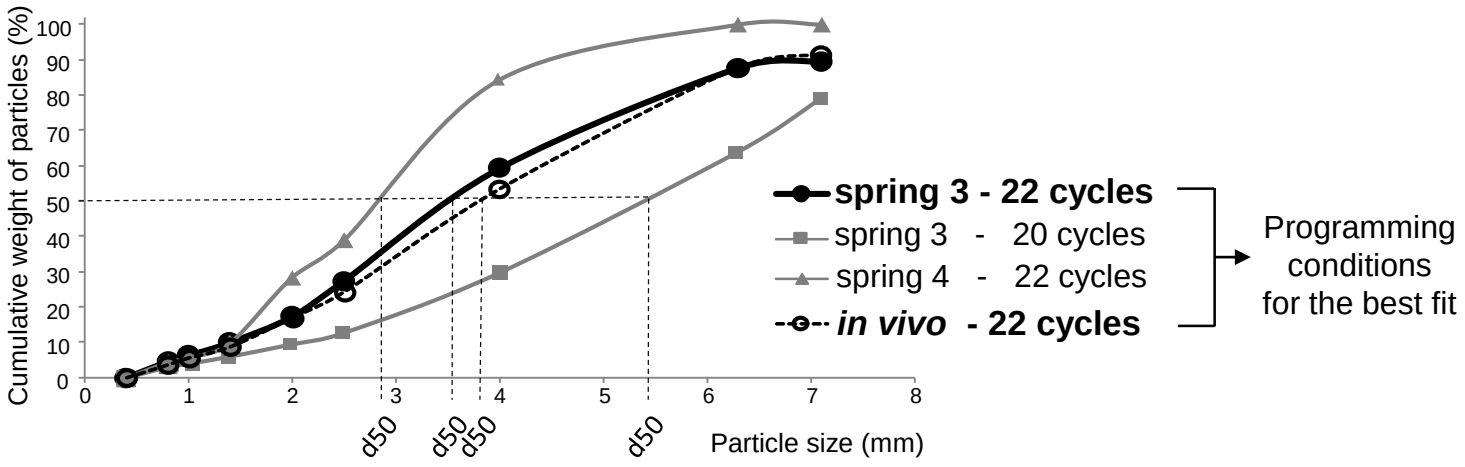

Cumulative weight of particles (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
d50
d50
d50
d50
Particle size (mm)
spring 3 - 22 cycles
spring 3 - 20 cycles
spring 4 - 22 cycles
in vivo - 22 cycles
Programming conditions for the best fit

Figure 2

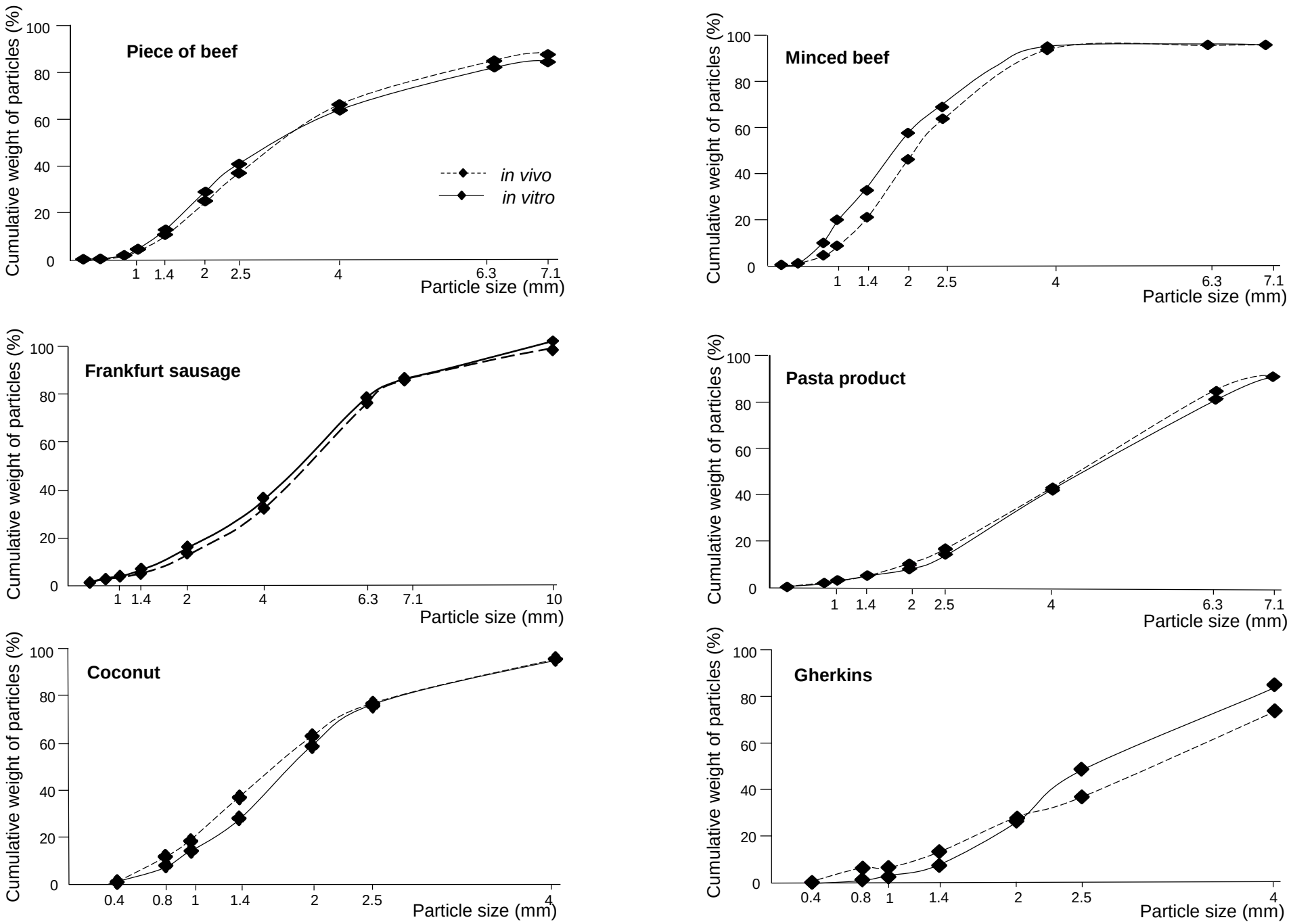

Piece of beef
Minced beef
Frankfurt sausage
Pasta product
Coconut
Gherkins
in vivo
in vitro
Cumulative weight of particles (%)
Particle size (mm)
0
20
40
60
80
100
0.4
0.8
1
1.4
2
2.5
4
6.3
7.1
10

Figure 3

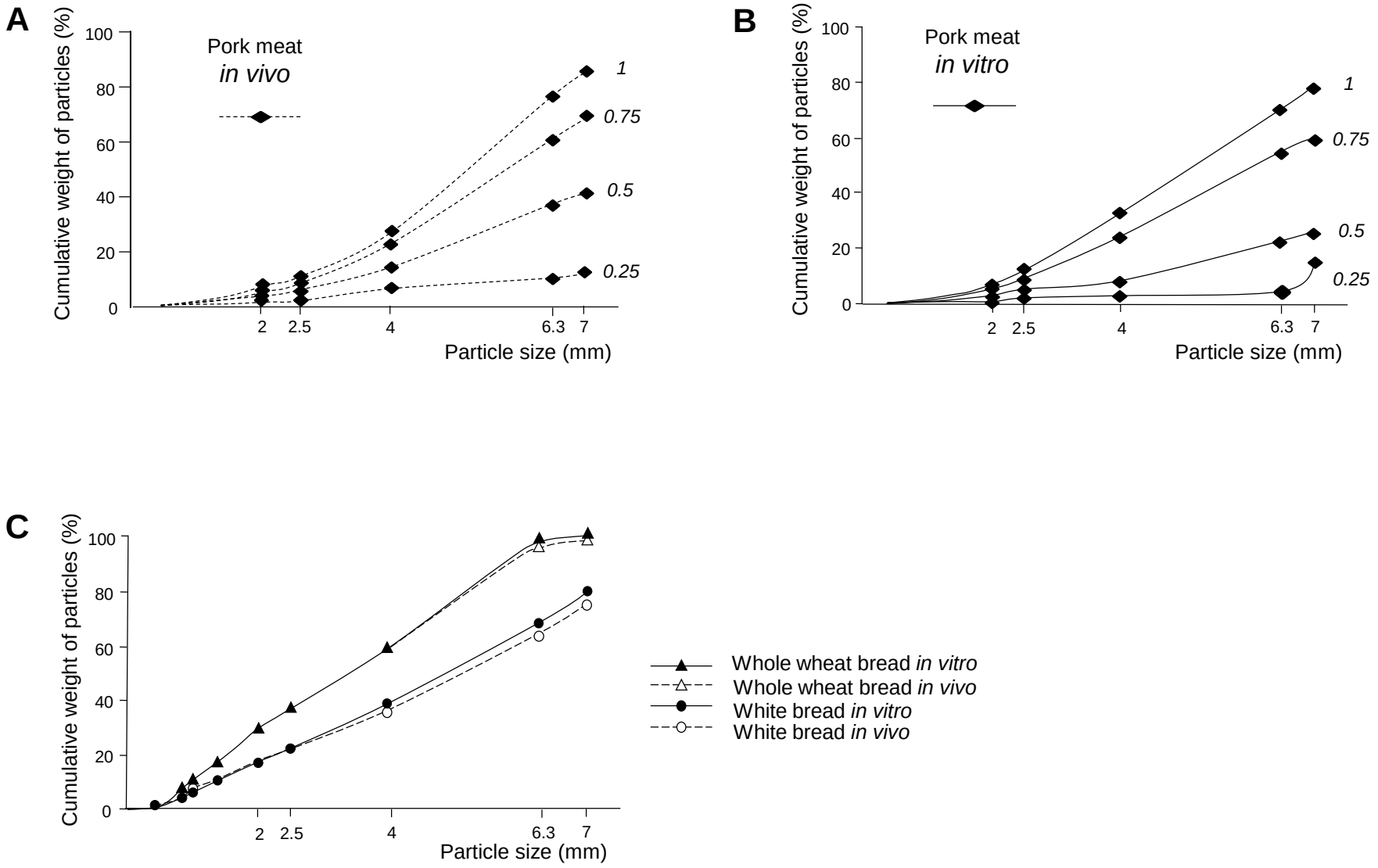

A
Pork meat
in vivo
Cumulative weight of particles (%)
100
80
60
40
20
0
1
0.75
0.5
0.25
2
2.5
4
6.3
7
Particle size (mm)
B
Pork meat
in vitro
Cumulative weight of particles (%)
100
80
60
40
20
0
1
0.75
0.5
0.25
2
2.5
4
6.3
7
Particle size (mm)
C
Cumulative weight of particles (%)
100
80
60
40
20
0
2
2.5
4
6.3
7
Particle size (mm)
Whole wheat bread in vitro
Whole wheat bread in vivo
White bread in vitro
White bread in vivo

Figure 4

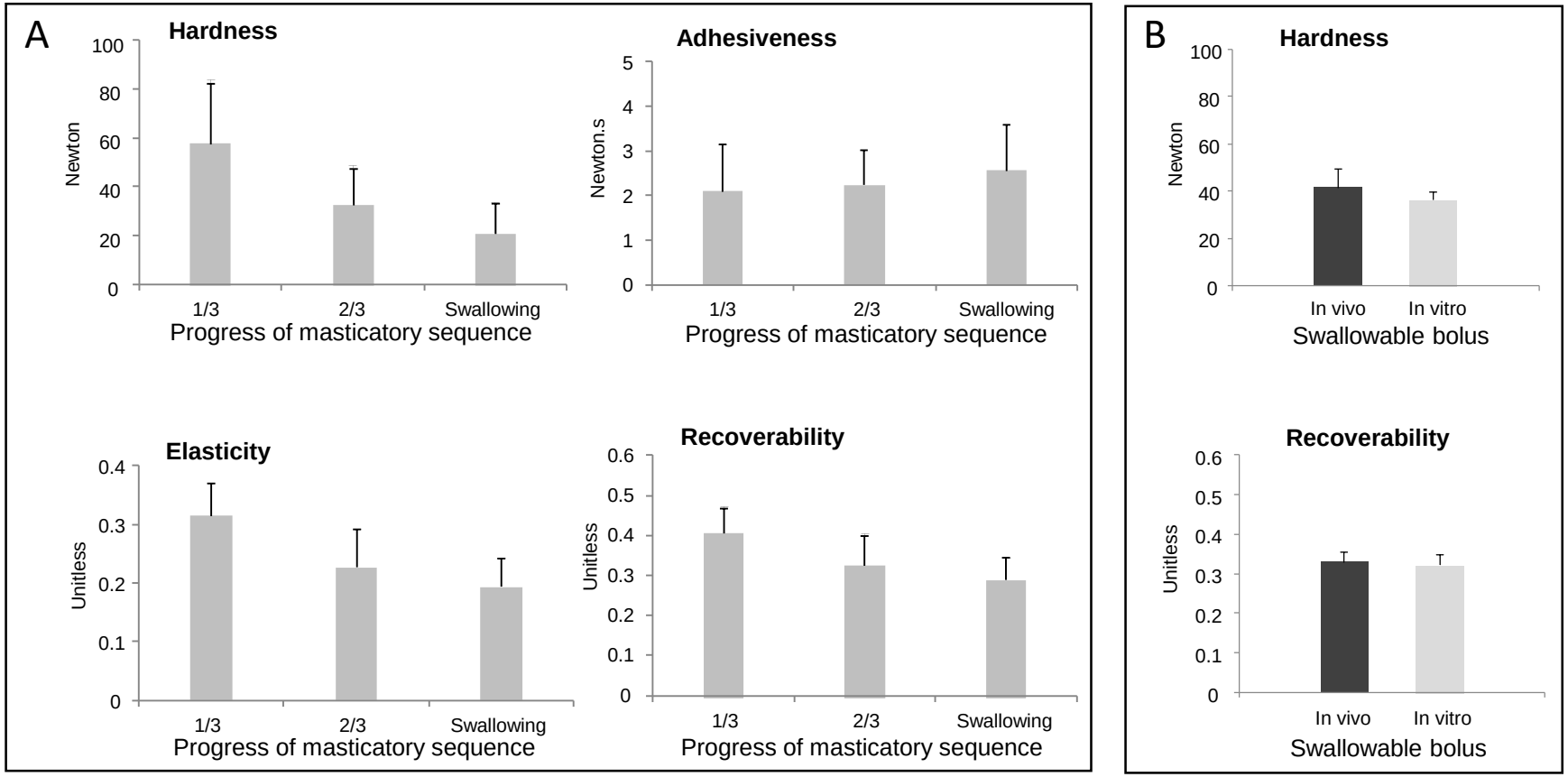

A
Hardness
Newton
100
80
60
40
20
0
1/3
2/3
Swallowing
Progress of masticatory sequence
Adhesiveness
Newton.s
5
4
3
2
1
0
1/3
2/3
Swallowing
Progress of masticatory sequence
Elasticity
Unitless
0.4
0.3
0.2
0.1
0
1/3
2/3
Swallowing
Progress of masticatory sequence
Recoverability
Unitless
0.6
0.5
0.4
0.3
0.2
0.1
0
1/3
2/3
Swallowing
Progress of masticatory sequence
B
Hardness
Newton
100
80
60
40
20
0
In vivo
In vitro
Swallowable bolus
Recoverability
Unitless
0.6
0.5
0.4
0.3
0.2
0.1
0
In vivo
In vitro
Swallowable bolus

Figure 5

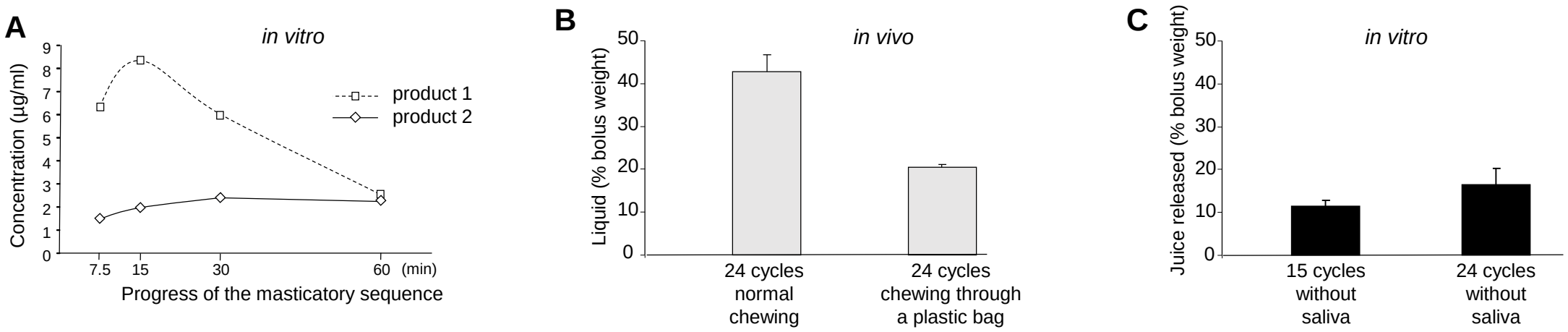
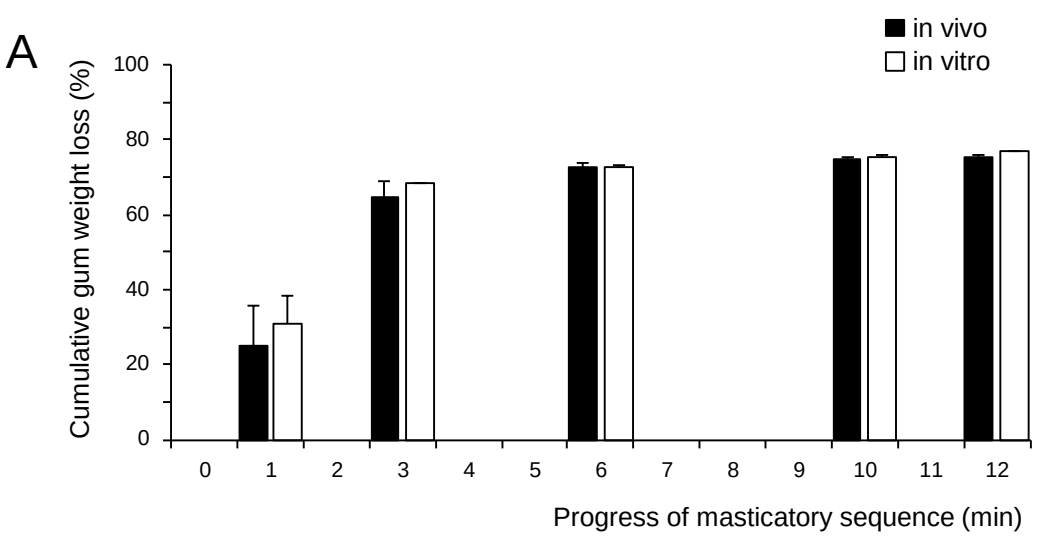

A
in vitro
Concentration (µg/ml)
9
8
7
6
5
4
3
2
1
0
product 1
product 2
7.5
15
30
60
(min)
Progress of the masticatory sequence
B
in vivo
Liquid (% bolus weight)
50
40
30
20
10
0
24 cycles normal chewing
24 cycles chewing through a plastic bag
C
in vitro
Juice released (% bolus weight)
50
40
30
20
10
0
15 cycles without saliva
24 cycles without saliva

Figure 6


A
in vivo
in vitro
Cumulative gum weight loss (%)
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Progress of masticatory sequence (min)

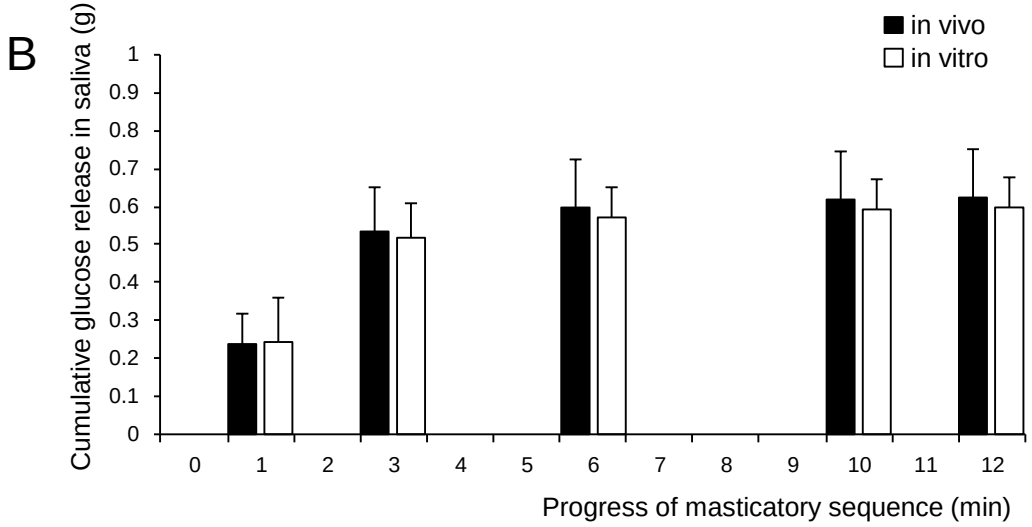

B
in vivo
in vitro
Cumulative glucose release in saliva (g)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Progress of masticatory sequence (min)

Figure 7

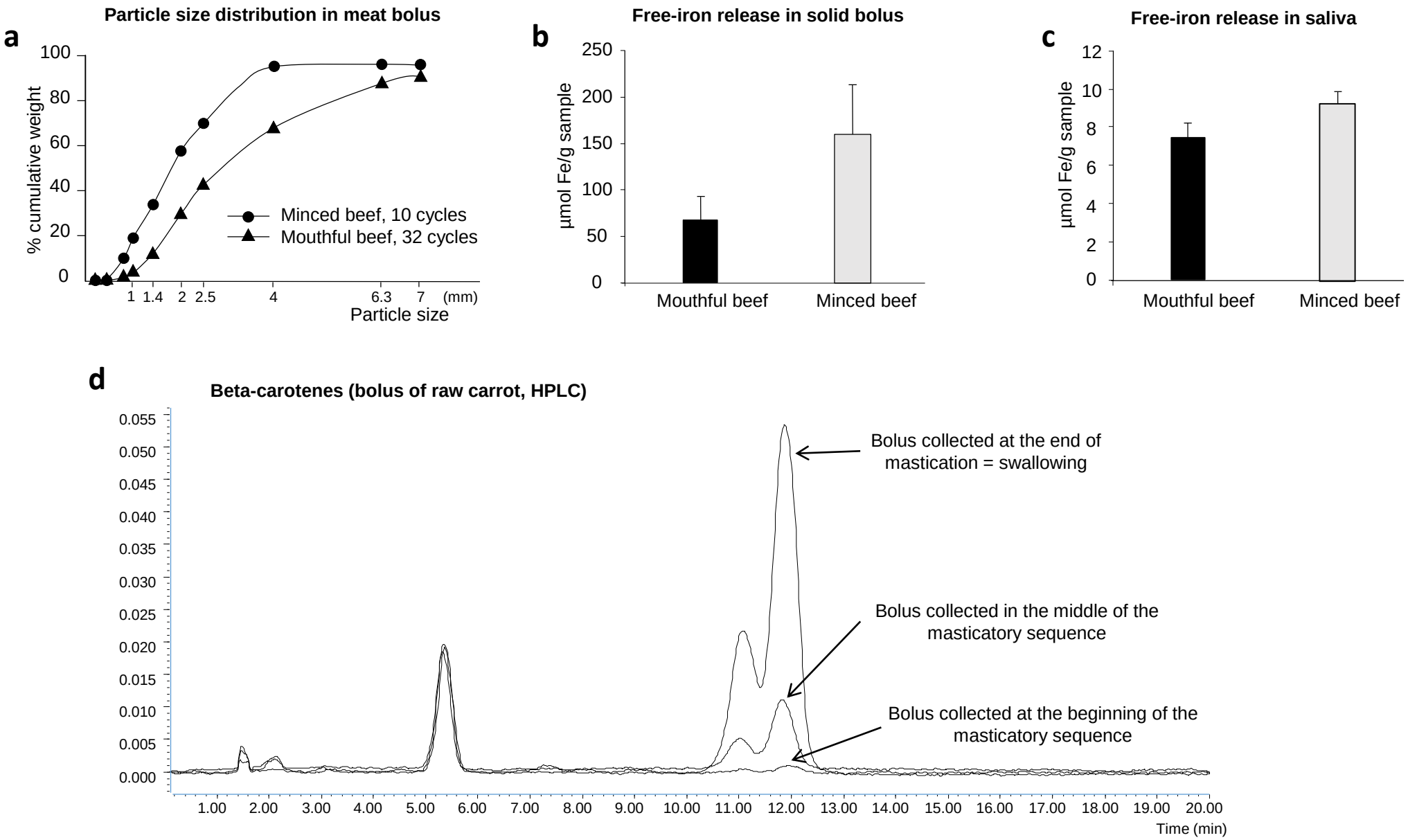

a
Particle size distribution in meat bolus
% cumulative weight
100
80
60
40
20
0
1
1.4
2
2.5
4
6.3
7
(mm)
Particle size
Minced beef, 10 cycles
Mouthful beef, 32 cycles
b
Free-iron release in solid bolus
µmol Fe/g sample
250
200
150
100
50
0
Mouthful beef
Minced beef
c
Free-iron release in saliva
µmol Fe/g sample
12
10
8
6
4
2
0
Mouthful beef
Minced beef
d
Beta-carotenes (bolus of raw carrot, HPLC)
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
Time (min)
Bolus collected at the end of mastication = swallowing
Bolus collected in the middle of the masticatory sequence
Bolus collected at the beginning of the masticatory sequence

Figure 8

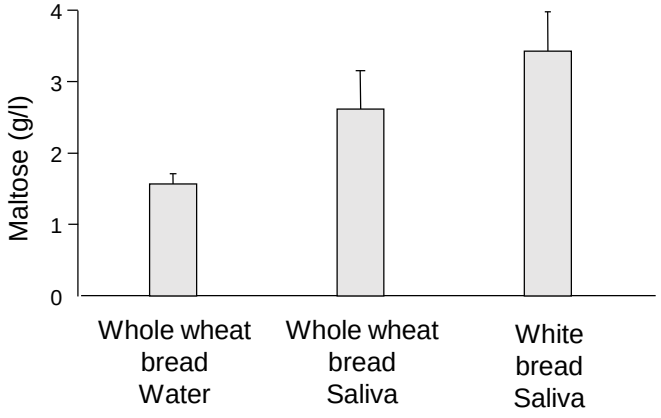
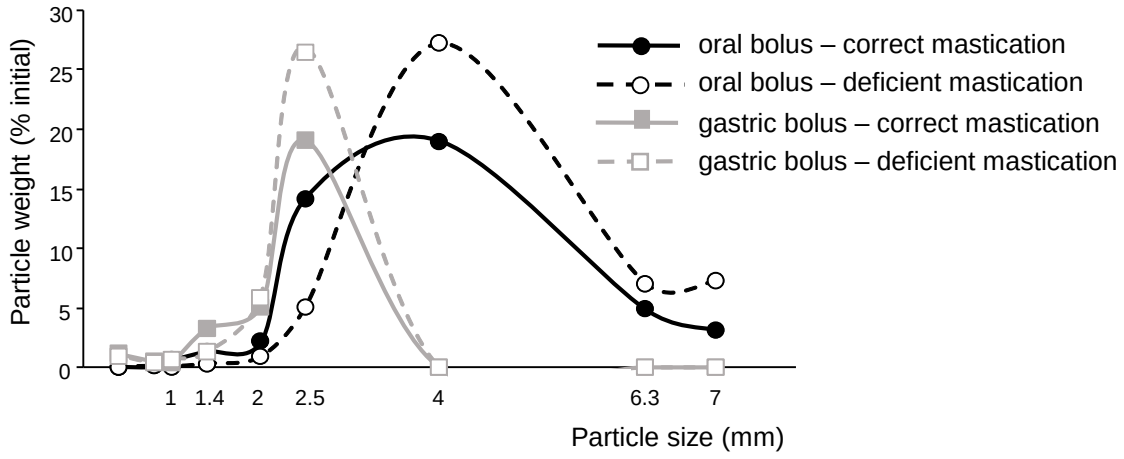

Maltose (g/l)
4
3
2
1
0
Whole wheat bread Water
Whole wheat bread Saliva
White bread Saliva

Figure 9

A


Particle weight (% initial)
30
25
20
15
10
5
0
1
1.4
2
2.5
4
6.3
7
Particle size (mm)
oral bolus – correct mastication
oral bolus – deficient mastication
gastric bolus – correct mastication
gastric bolus – deficient mastication

B

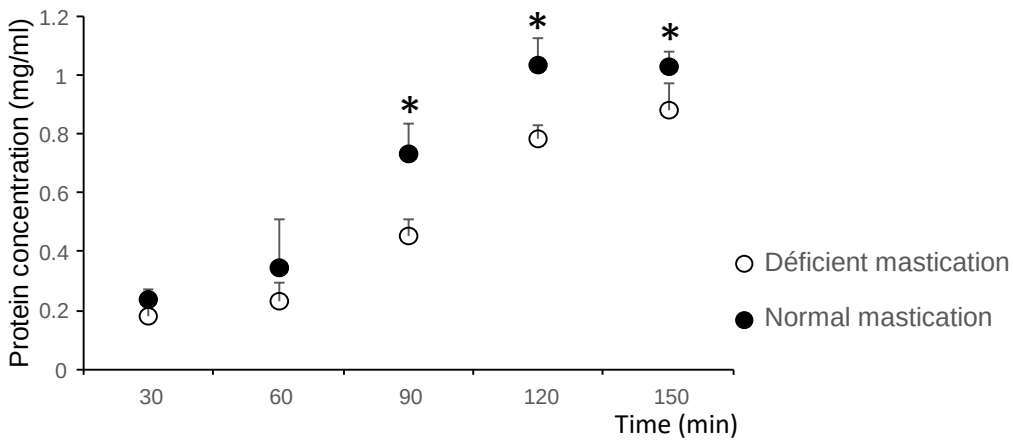

Protein concentration (mg/ml)
1.2
1
0.8
0.6
0.4
0.2
0
30
60
90
120
150
Time (min)
*
*
*
Déficient mastication
Normal mastication

Figure 10

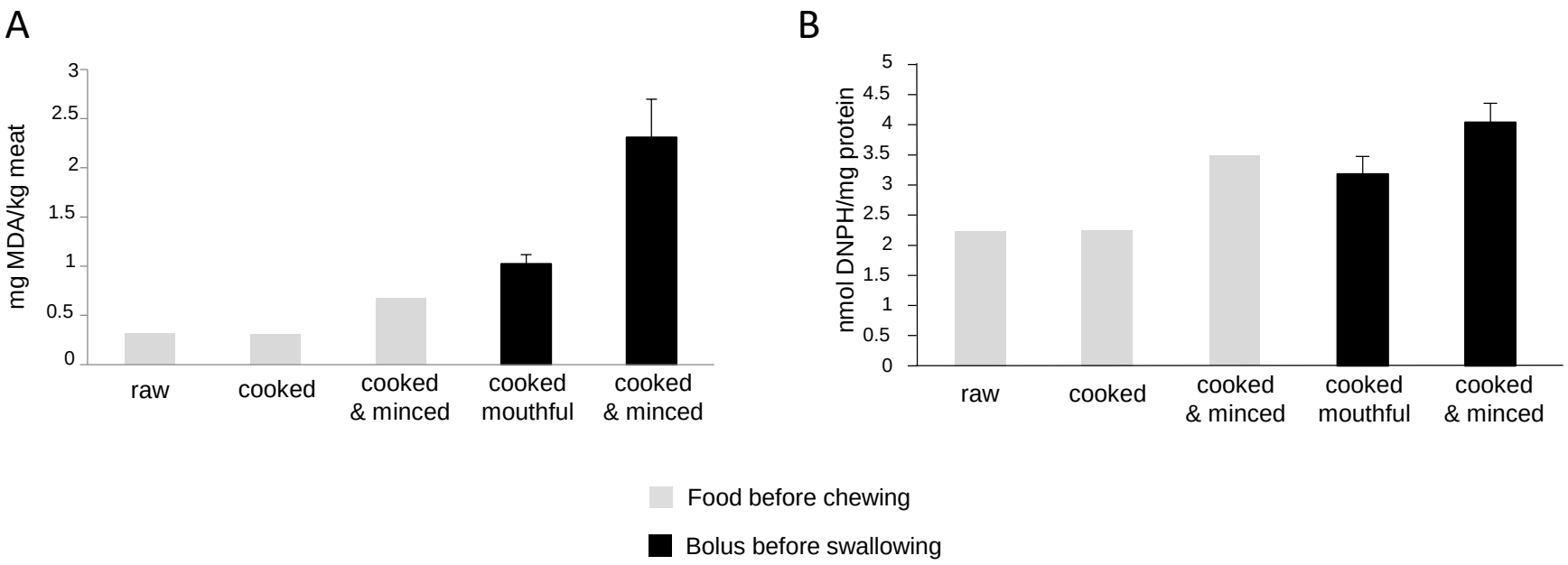

A
mg MDA/kg meat
3
2.5
2
1.5
1
0.5
0
raw
cooked
cooked & minced
cooked mouthful
cooked & minced
B
nmol DNPH/mg protein
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
raw
cooked
cooked & minced
cooked mouthful
cooked & minced
Food before chewing
Bolus before swallowing

Figure 11

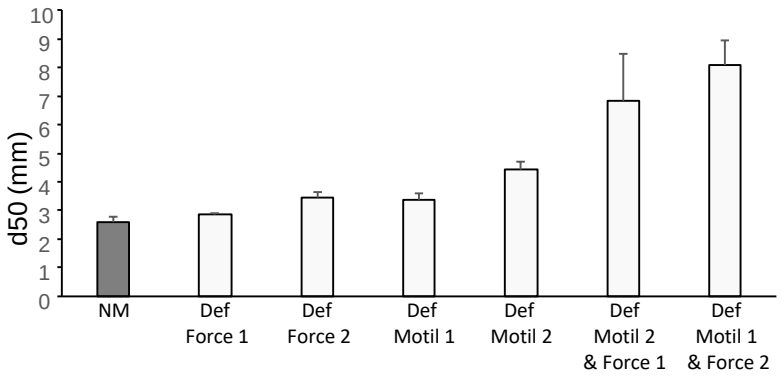

d50 (mm)
10
9
8
7
6
5
4
3
2
1
0
NM
Def Force 1
Def Force 2
Def Motil 1
Def Motil 2
Def Motil 2 & Force 1
Def Motil 1 & Force 2

Figure 12

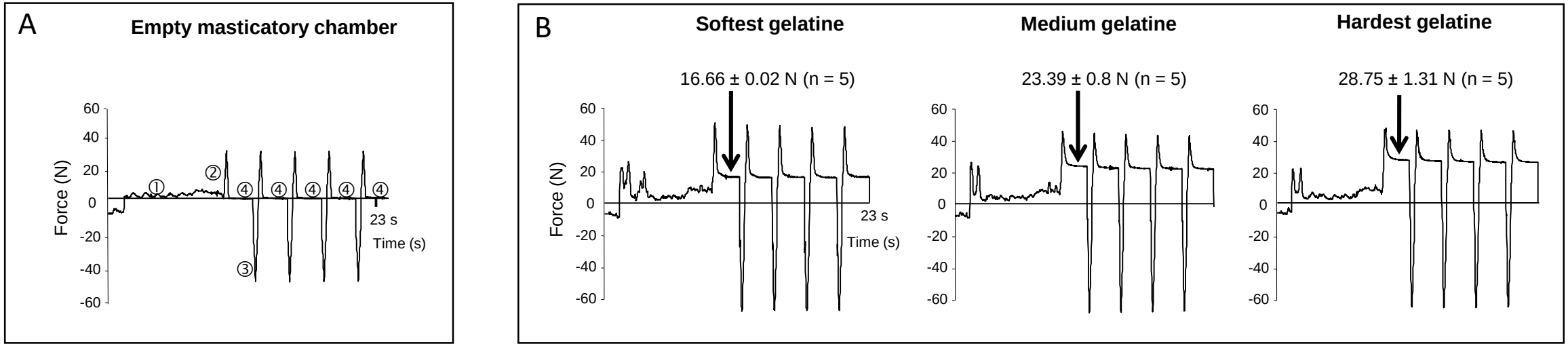
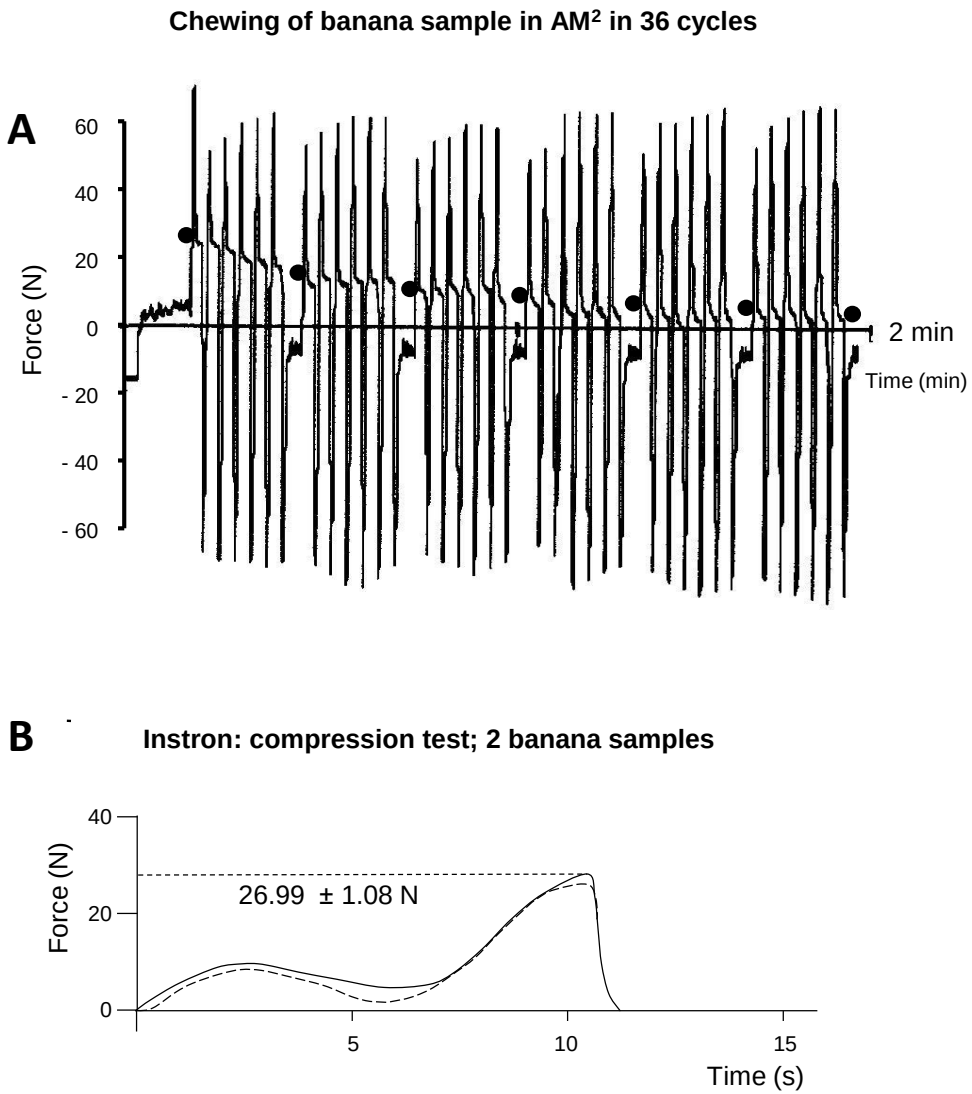

A
Empty masticatory chamber
Force (N)
60
40
20
0
-20
-40
-60
①
②
③
④
23 s
Time (s)
B
Softest gelatine
16.66 ± 0.02 N (n = 5)
Medium gelatine
23.39 ± 0.8 N (n = 5)
Hardest gelatine
28.75 ± 1.31 N (n = 5)

Figure 13


Chewing of banana sample in AM² in 36 cycles
A
Force (N)
60
40
20
0
- 20
- 40
- 60
2 min
Time (min)
B
Instron: compression test; 2 banana samples
Force (N)
40
20
0
26.99 ± 1.08 N
5
10
15
Time (s)

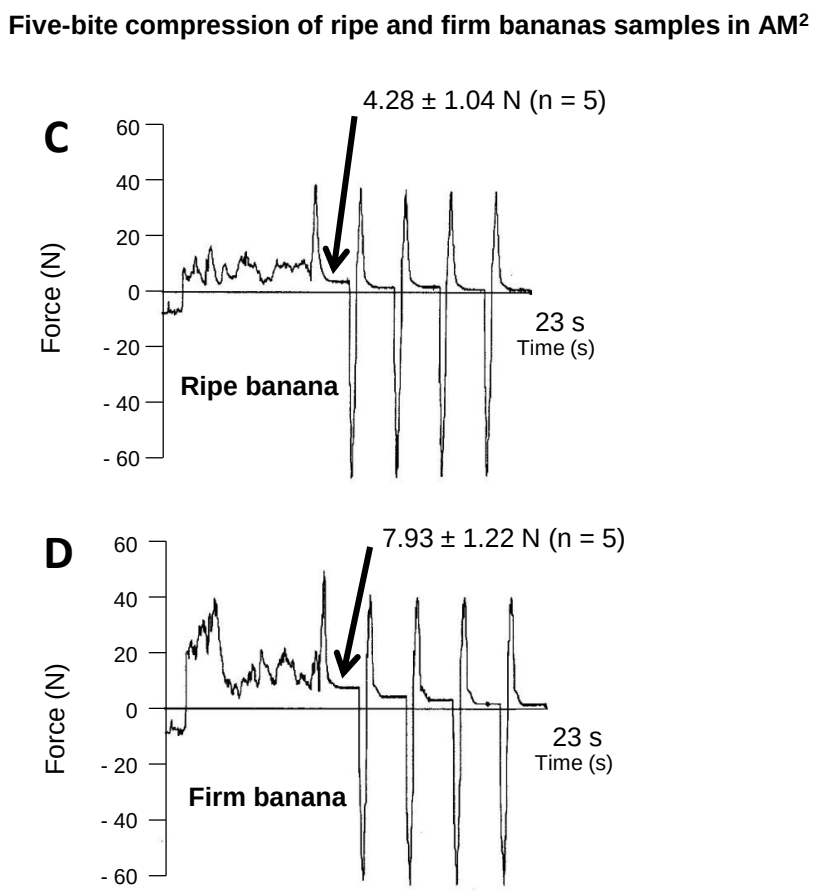
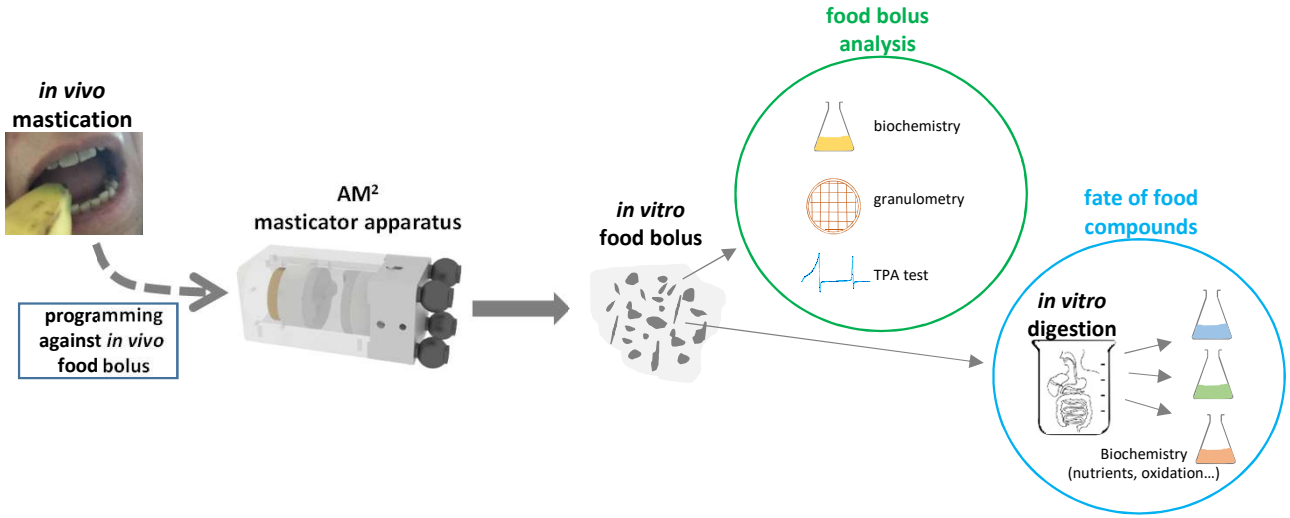

Five-bite compression of ripe and firm bananas samples in AM²
C
4.28 ± 1.04 N (n = 5)
Force (N)
60
40
20
0
- 20
- 40
- 60
23 s
Time (s)
Ripe banana
D
7.93 ± 1.22 N (n = 5)
Force (N)
60
40
20
0
- 20
- 40
- 60
23 s
Time (s)
Firm banana

Figure 14


in vivo
mastication
programming against in vivo food bolus
AM²
masticator apparatus
in vitro
food bolus
food bolus analysis
biochemistry
granulometry
TPA test
fate of food compounds
in vitro
digestion
Biochemistry
(nutrients, oxidation…)