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1 ADDRESSING VARIOUS CHALLENGES RELATED TO FOOD BOLUS AND  
2 NUTRITION WITH THE AM<sup>2</sup> MASTICATION SIMULATOR

3

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**26 Abstract**

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

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

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

46

47

**48 Keywords**

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

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**53 Highlights**

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

59

60

**61 1. Introduction**

62

63 Food oral processing and more specifically the ready-to-swallow food bolus have become  
64 major topics of interest in food and dental science (Engelen, Fontijn-Tekamp, & Bilt, 2005)  
65 and in the field of nutrition (Bonnet, Batisse, Peyron, Nicolas, & Hennequin, 2018; Chen,  
66 2009, 2015; Engelen, Fontijn-Tekamp, & Bilt, 2005; Ng et al., 2017). Food bolus analysis is  
67 at the crossroads between food structure (Gao, Wong, Lim, Henry, & Zhou, 2015), food  
68 formulation, the oral perception of food, food oral processing (Hwang et al., 2012; Jalabert-  
69 Malbos, Mishellany-Dutour, Woda, & Peyron, 2007; Koç et al., 2014; Shiozawa, Kohyama,  
70 & Yanagisawa, 2003) and the further stages of digestion (Hoebler, Devaux, Karinhi,  
71 Belleville, & Barry, 2000; Hoebler et al., 1998), including oral digestion and related nutrient  
72 bioaccessibility (Ellis et al., 2004; Grundy et al., 2015). However, the mere fact that food  
73 bolus formation occurs inside the mouth and that access inside the mouth is not possible  
74 during mastication hampers most study designs. Collecting the ready-to-swallow bolus in  
75 normal or deficient subjects is not a simple procedure. Among other reasons, the bolus is

76 often partly swallowed before the final swallow event (Hiemae et al., 1996), the bolus itself  
77 is mixed in an unknown proportion with saliva, making it impossible to determine the  
78 proportion of saliva, food fluids and solid nutrients present within the bolus during the  
79 masticatory process. During chewing, several physico-chemical processes are at work,  
80 transforming the characteristics of the final bolus in an uncontrolled way. In addition, asking  
81 volunteers to masticate is sometimes impossible for several reasons such as the use of  
82 “experimental” or non-natural food, the presence of food contaminants, the chewers studied  
83 are disabled, young children or elderly people, or simply because it is too time consuming.  
84 Therefore, *in vitro* mastication using a mastication simulator is a valuable alternative to an *in*  
85 *vivo* approach for the experimental production and analysis of food boluses. This kind of  
86 simulator also provides solutions for multiple analyzes of food bolus properties at the time of  
87 swallowing or of the kinetics involved during its formation, and it provides a more realistic  
88 oral simulation than that frequently ~~done~~ performed before (Funami, 2016; Hedrén, Diaz, &  
89 Svanberg, 2002; Hoebler et al., 2002; Minekus et al., 2014). The possible questions of interest  
90 include saliva impregnation, the oral release of nutrients and oral digestion assessment,  
91 investigations on the impact of food formulation on the food bolus and its characteristics, for  
92 example, in the design of new foods for targeted populations (the elderly, children, non-  
93 cooperative human subjects, and even animals). It can also be used to examine the oral release  
94 of nutrients and of active, toxic substances and food contaminants from the matrix being  
95 chewed, for example, to evaluate the role of the oral cavity in the balance between the oral  
96 bioaccessibility and bioavailability of nutrients. Using a mastication simulator is also very  
97 useful for the production of specific food boluses to address the fate of the food in the  
98 digestive tract. Numerous studies are conducted with a simulated bolus produced by mincing,  
99 mixing or crushing a food sample without any control of particle size distribution and saliva  
100 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 destructurement 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<sup>2</sup> 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<sup>2</sup>) 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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> masticator apparatus could be produced to  
133 demand. Designing modified prototypes is also undoubtedly a realistic goal in view to  
134 satisfying specific needs to answer various potential scientific questions.

135

## 136 **2. The masticator apparatus AM<sup>2</sup>**

137

### 138 *2.1 Description of the masticatory apparatus AM<sup>2</sup>*

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

151 during mastication depends on the stiffness of the spring which is chosen as a function of food  
152 resistance (Mishellany-Dutour et al., 2011; Woda et al., 2010a). The actions of shearing food  
153 material, as well as gathering particles and transporting them to the masticatory surface are  
154 performed by the rotational movement of the mandibular disk, as the tongue would do. Water  
155 or a validated artificial saliva (Roger-Leroi, Mishellany-Dutour, Woda, Marchand, & Peyron,  
156 2012) can be injected at the beginning and / or by staggered injections during the masticatory  
157 sequence.

158

## 159 *2.2 Programming of the masticatory apparatus AM<sup>2</sup>*

160

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

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

176 experimental tests, the mastication of a French baguette sample would be done with the 10.38  
177 N/mm spring and a raw carrot sample with the 25 N/mm spring. The number of masticatory  
178 cycles is always obtained from the *in vivo* measurements of mastication of the food  
179 considered. A large angle (around 200°) is chosen for a “fibrous” food, such as meat needing  
180 more shear stress to be disrupted compared to a brittle food such as cookies for which an  
181 angle of 90° is enough. Various rotational angles are also tested for gathering particles. The  
182 temperature of the masticatory chamber can be regulated at 36°C if the oral processing  
183 studied is temperature dependent. Water, human or artificial saliva can be used for  
184 mastication. The flow and composition of artificial saliva with respect to mucin and enzyme  
185 contents (amylase and lipase, for example) can be chosen according to the scientific issue to  
186 be addressed (Roger-Leroi et al., 2012). Fig. 2 shows an example of the programming  
187 approach in which two numbers of masticatory cycles and two levels of spring-induced forces  
188 were sequentially changed in the programming of the masticator apparatus to find the best fit  
189 with the *in vivo* particle size distribution curve in meat bolus analyzed at swallowing time. As  
190 shown in Fig. 2, by performing the same number of masticatory cycles as observed *in vivo*,  
191 the stiff spring (23.5 N/mm) overly-disrupted the meat bolus compared to the softer spring  
192 (18.2 N/mm). Finally, after progressive adjustment, the mean particle size distribution curves  
193 of the meat boluses obtained *in vivo* and *in vitro* in the conditions selected were identical at  
194 swallowing time (Fig. 2).

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

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

202 A complete validation of the apparatus should also be performed by considering other  
203 physical properties of the food bolus, for example, using an instrumental Texture Profile  
204 Analysis (TPA) test consisting in applying double compression on the food bolus. Despite its  
205 empirical nature, this test is useful to compare the properties of food boluses collected at  
206 different times of the masticatory sequence, for different food matrixes or in different oral  
207 conditions, both for *in vivo* or *in vitro* food boluses (Peyron et al., 2011). Hardness,  
208 recoverability, adhesiveness and springiness values can be extracted from each TPA curve for  
209 bolus mechanical characterization. As an example of this interesting use, Fig. 5A illustrates  
210 that a piece of French bread (baguette) is softened, rendered less elastic and less cohesive as  
211 the *in vivo* masticatory sequence progresses. Physical characteristics obtained for *in vivo* and  
212 *in vitro* boluses can be compared to attest the effectiveness of the masticator apparatus (Fig.  
213 5B). The possible addition of water, or human or artificial saliva in the AM<sup>2</sup> is undoubtedly a  
214 key parameter in preparing a food bolus for instrumental measurements since it provides the  
215 specific rheological environment of the food after mastication. A comparison of the physical  
216 characteristics of the *in vitro* food boluses obtained with these different fluids would be  
217 informative regarding the role of the specific components such as enzymes and proteins.  
218 Nevertheless, great care must be taken in the treatment of TPA test variables which are not  
219 absolute values, they are often misinterpreted, and they can only be used in a comparative  
220 way (Nishinari & Fang, 2018).

221 All the *in vivo* data used to validate or to program the masticator apparatus were obtained  
222 after collecting the informed consent of each subject and experiments were performed in  
223 accordance with different local and national ethical agreements (CE-CIC-GREN-10/06-  
224 #5044; DREAM-2013-A00096-39).

225

### 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 (Hiemae & Palmer,  
248 1999). AM<sup>2</sup> 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<sup>2</sup> (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<sup>2</sup> 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<sup>2</sup> apparatus.

275

#### 276 4. Oral bioaccessibility of nutrients, digestion studies

277

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

298 Fig. 9 illustrates the first step of digestion occurring in the mouth during mastication and  
299 quantified by the production of maltose after the oral hydrolysis of starch by salivary amylase.  
300 Pieces of different breads (2.5 g) were introduced in the AM<sup>2</sup>. 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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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<sup>2</sup> 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

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

382

### 383 **7. Measurement of forces applied during mastication**

384

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

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

401 with the Instron machine (Fig. 14B) performing a single compression test at 65% deformation  
402 (the same deformation range applied on the banana sample by the AM<sup>2</sup>) on two banana  
403 samples taken from the same bunch as the sample used for the experiment presented in Fig.  
404 14A. The resistance force obtained with the Instron machine when the sample was  
405 compressed ( $26.99 \pm 1.08\text{N}$ ) was comparable to that obtained with the AM<sup>2</sup> at the first bite. In  
406 another experiment presented in Fig. 14 (C and D), the AM<sup>2</sup> was used to mimick testing  
407 machine mode to test the effect of fruit ripening. The ripe and firm banana samples were  
408 subjected to five successive compressions. The resistance was higher ( $7.93 \pm 1.22\text{N}$ ) for the  
409 firm banana than for the ripe one ( $4.28 \pm 1.04\text{N}$ ).

410

## 411 **8. Conclusion**

412

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

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

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

429

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436

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438

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638

639

## 640 **Legends**

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

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

656 Figure 3: Comparisons between mean particle size distribution curves of boluses obtained *in*  
657 *vivo* and *in vitro* from different food types. Piece of beef: cooked samples, 2cm square and  
658 1cm thick. Minced beef: same cooked meat, molded in the same sample shape as non-minced  
659 meat. Frankfurt sausage: cylindrical samples of 6.5g. Pasta products: cooked Fusilli pasta in  
660 7g bite. Coconut: 1cm square and 1cm thick sample. Gherkins: cylindrical sample of 3.5g.  
661 Each point represents the weight of the particles (in %) retained in the corresponding sieve  
662 size added to the sum of all the preceding points (cumulative curve). The similarity required  
663 between *in vivo* and *in vitro* bolus granulometry was not limited to the median particle size  
664 (*d50=50% on cumulative curve*) but sought for the whole mean particle size distribution  
665 curves. In these examples, better fitting could be obtained for minced meat and gherkins if  
666 further studies were carried out. For the sake of readability, standard deviations are not drawn.

667 Figure 4: Comparisons between mean particle size distribution curves for boluses obtained *in*  
668 *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 ( $\emptyset$  28 mm) moved at a constant  
682 displacement rate of 50 mm.min<sup>-1</sup>, a cylindrical container (int.  $\emptyset$  35 mm, ext.  $\emptyset$  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<sup>2</sup>.

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<sup>2</sup> (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<sup>2</sup> (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 ( $\emptyset$  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<sup>2</sup> 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

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

747 Figure 14. Forces recorded with the strength sensor of the AM<sup>2</sup> (Fig. 1) while chewing banana  
748 samples with 1.8 ml saliva, at 100 mm/s translational speed. A: Successive bites performed in  
749 2 minutes chewing. The progressively decreasing force—from  $25.42 \pm 0.81\text{N}$  to almost zero, is  
750 highlighted by black circles and illustrates that the banana sample was progressively crushed  
751 and less resistant with the successive bites. B: Measures with an Instron machine performing  
752 a single compression test in replicates on 2 banana samples (2 cm diameter, 1 cm high) with a  
753 cylindrical piston head of 2.8 cm diameter. The values of forces in Newtons ( $26.99 \pm 1.08\text{ N}$ )  
754 were similar to the value obtained during the first bite with AM<sup>2</sup> ( $25.42 \pm 0.81\text{ N}$ ). C and D:  
755 Measures of forces developed during a five-bite compression to test the effect of banana  
756 ripening on sample resistance with the AM<sup>2</sup> mimicking a testing machine mode (no saliva,  
757 spring of lowest stiffness, mobile **disk** actuated at 100mm/s).



## PROOFREADING CERTIFICATE

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

**ADDRESSING VARIOUS CHALLENGES RELATED TO FOOD BOLUS AND NUTRITION**

**WITH THE AM<sup>2</sup> MASTICATION SIMULATOR**

was proofread and corrected by a native English speaker working for Accent Europe on 13 June 2019.

Articles proofread or translated by Accent Europe have been published in the following journals:

<p><b>Agronomy</b> Cereals European Journal of Agronomy European Journal of Soil Science Geoderma Pedologia Plants and Soil Soil and Tillage Research Soil Science Society of America</p> <p><b>Biology</b> Cell Fundamental and Applied Limnology Global Change Biology Hydrobiologia International Journal of Limnology Journal of Clinical Microbiology Mutation Research - Genetic Toxicology and Environmental Mutagenesis</p> <p><b>Chemistry</b> Analytical Chemistry Chemical engineering journal Chemical engineering science Journal of the American Chemical Society Kinetics and Catalysis Polymer Testing</p> <p><b>Cognitive sciences, ergonomics, health, medicine</b> Accident Analysis and Prevention Aerosol and Air Quality Research Aerosol Science and Technology Allergy American Journal of Epidemiology Annals of Occupational Hygiene Applied acoustics Applied ergonomics Archives of toxicology British Medical Journal Diabetes Disease-a-Month Environmental and Molecular Mutagenesis Ergonomics Filtration and separation Gerodontology Health Policy Injury Prevention International Journal of Audiology International Journal of Engineering Design International Journal of Hygiene and Environmental Health International Journal of Industrial Ergonomics International journal of occupational medicine and environmental health International Journal of Occupational Safety and Ergonomics International Journal of Pharmacokinetics International Journal of Ventilation International Society for Respiratory Protection JASA (Journal of Acoustical Society of America) Journal of Aerosol Materials</p>	<p>Journal of aerosol science Journal of Analytical Toxicology Journal of environmental and occupational hygiene Journal of environmental Hygiene Journal of Nanoparticle Research Journal of Occupational and Environmental Hygiene Journal of Pharmacological and Toxicological Methods Journal of Physics: Conference Series Journal of sound and vibration Neurotoxicology NT &amp; T Occupational and Environmental Medicine (OEM) Occupational Environmental Medicine Occupational Medicine Physiology &amp; Behaviour Powder technology Radiology Radioprotection Revue Thomas Safety Science The Journal of Infectious Diseases Scandinavian Journal of Work, Environment &amp; Health Springer Computer Science Toxicology and environmental chemistry Toxicology in vitro Traffic Injury Prevention Journal of oral rehabilitation Waste Management WDPF Pastry</p> <p><b>Computer Science and Mathematics</b> Computer &amp; Structures Computer Methods in Applied Mechanics and Engineering Computer methods in applied mechanics and engineering Computes and Information in Engineering Conference Computer and chemical engineering</p> <p><b>Civil Engineering</b> Canadian Journal of Civil Engineering Journal of Hydraulic Engineering European Journal of Environmental and Civil Engineering Hydropower and Dams Transport in Porous Media</p> <p><b>Economics</b> Economic Modelling International Journal of Public Sector Performance Labour International Journal of Transport Economics</p> <p><b>The environment</b> Actus-Lac - UNIBICO 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</p>	<p>Forest Ecology and Management Journal of Environmental Management Journal of Environmental Quality Journal of Hazardous Materials Marine Ecology Progress Series Microbial Ecology Sciences of the Total Environment Urban Water Journal Water Science and technology Journal of Hazardous Materials STOTEN Vadose Zone Journal Water Research</p> <p><b>Food Science</b> International Journal of Food Science and Technology</p> <p><b>Oceanography</b> Deep Sea Research Journal of the Marine Biological Association of the United Kingdom</p> <p><b>Structural mechanics, numerical simulation</b> ASME Journal of Applied Mechanics ASME Journal of Computational Methods and Nonlinear Dynamics ASME Journal of Vibration and Acoustics Comptes Rendus de Mécanique Computer Methods in Applied Mechanics and Engineering Granular Matter International Journal of Solids and Structures Journal of Applied Physics Journal of Heat Transfer Journal of Magnetism and Magnetic Materials Journal of Mechanical Design (ASME) Proceedings of the ASME 2012 Turbine Technical Conference and Exposition ASME, IMackE Journal of Mechanism and Machine Theory 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 Conference Proceedings of the ASME 2011 Turbine Technical Conference and Exposition The Shock and Vibration Bulletin Transactions of the ASME</p> <p><b>Tribology</b> Journal of Tribology Tribology International Wear</p>
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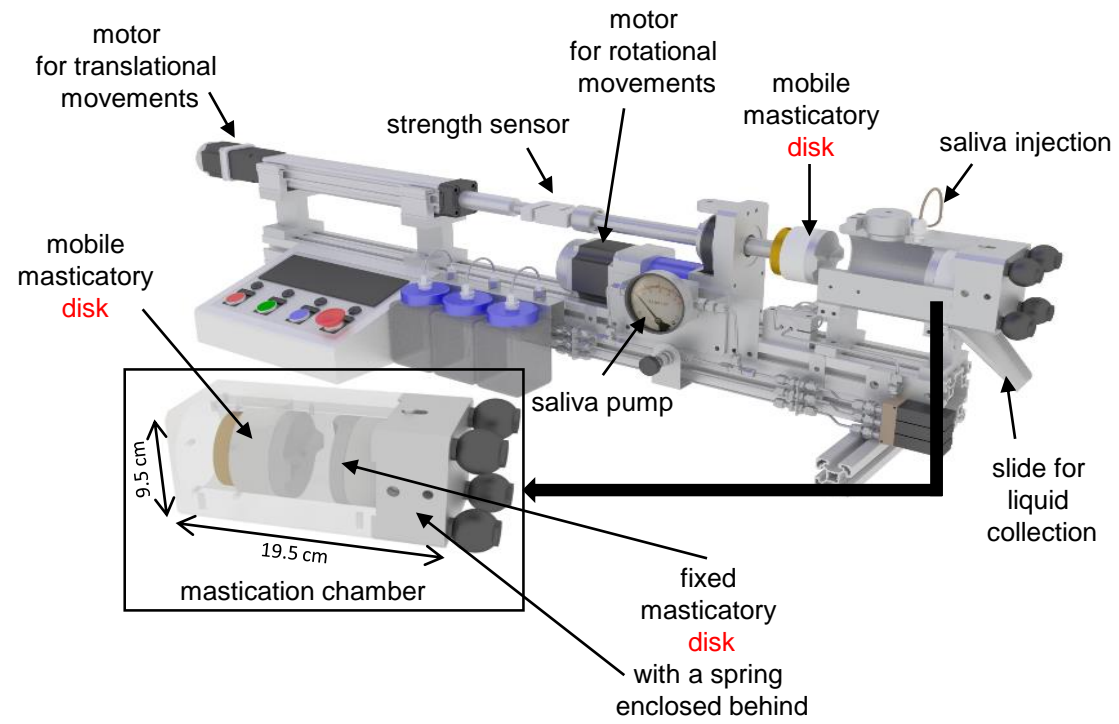


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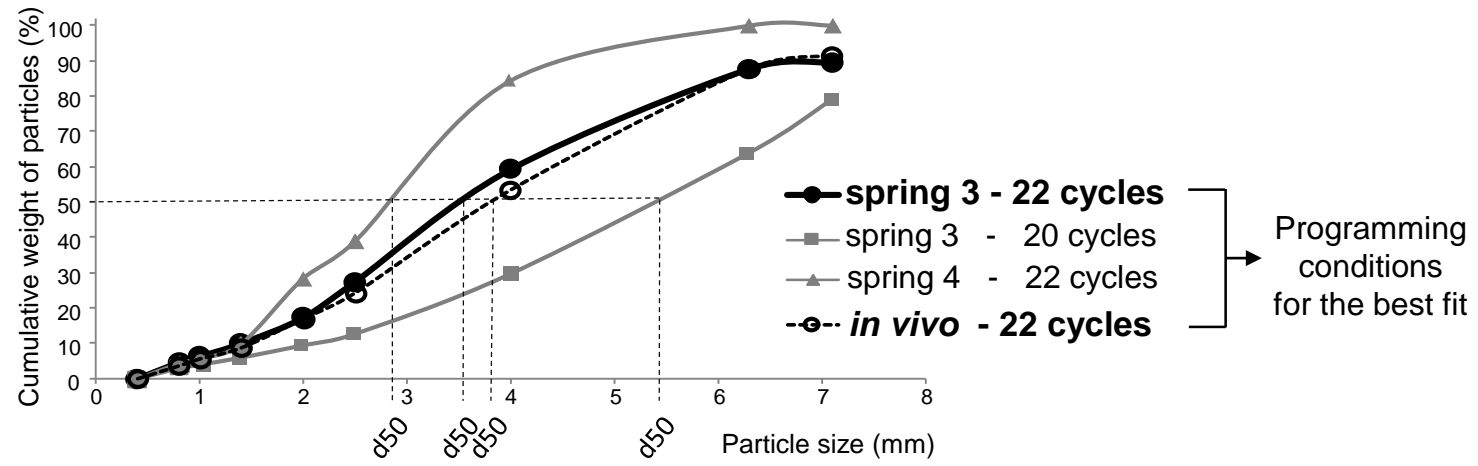


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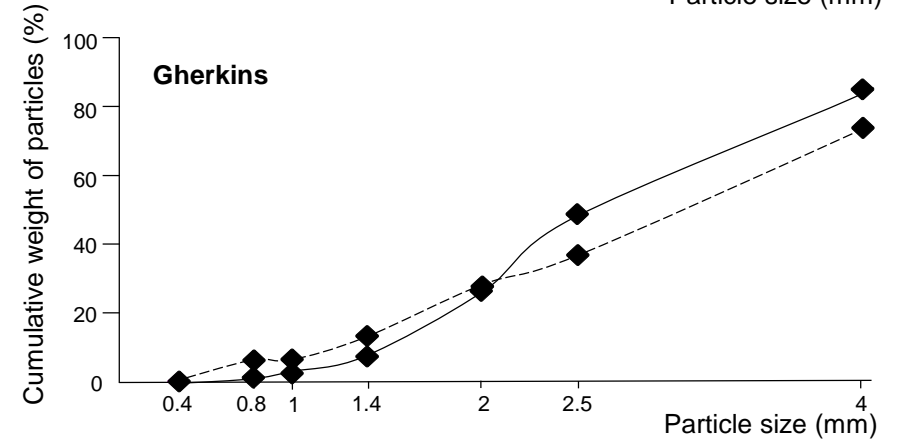
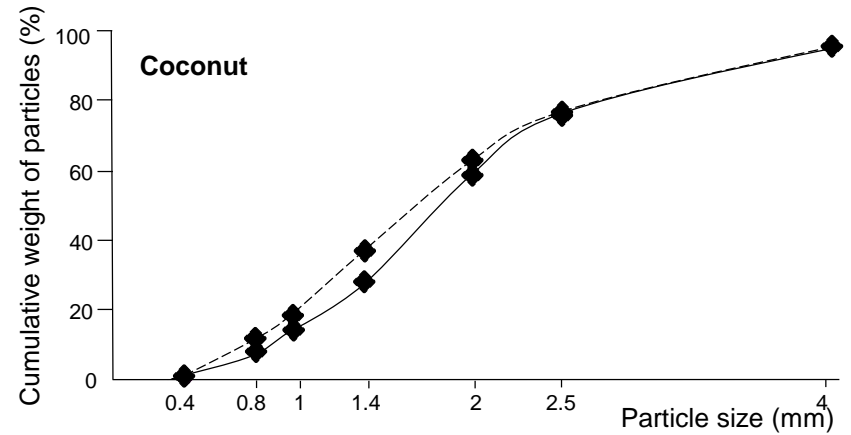
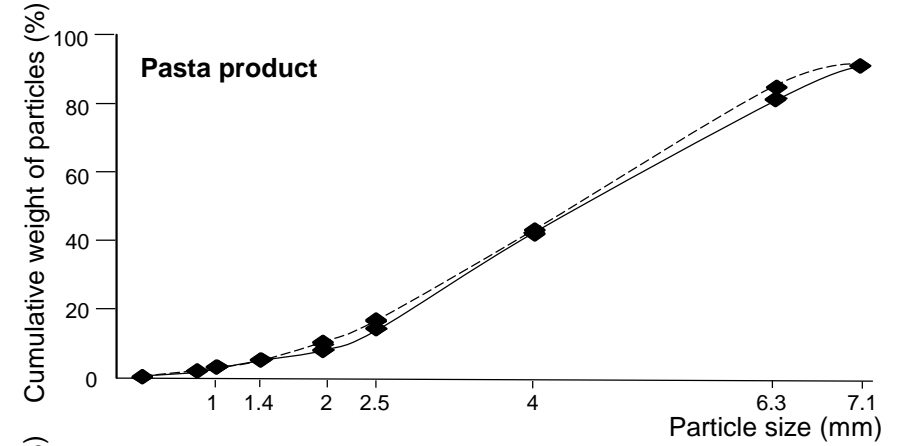
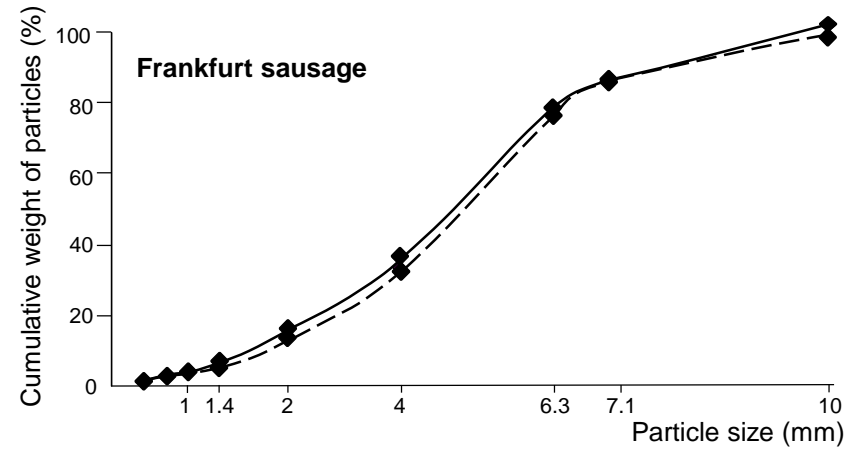
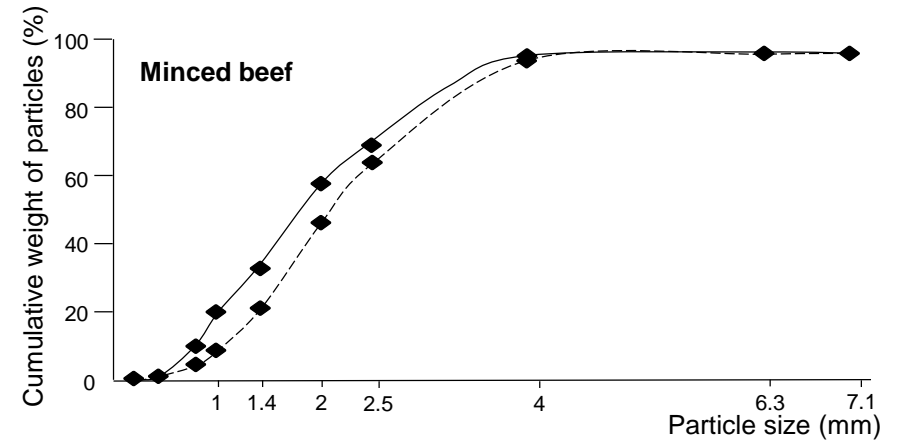
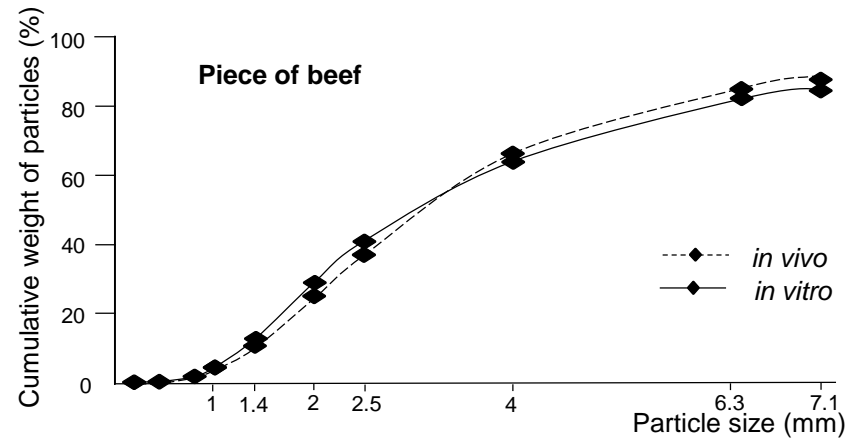


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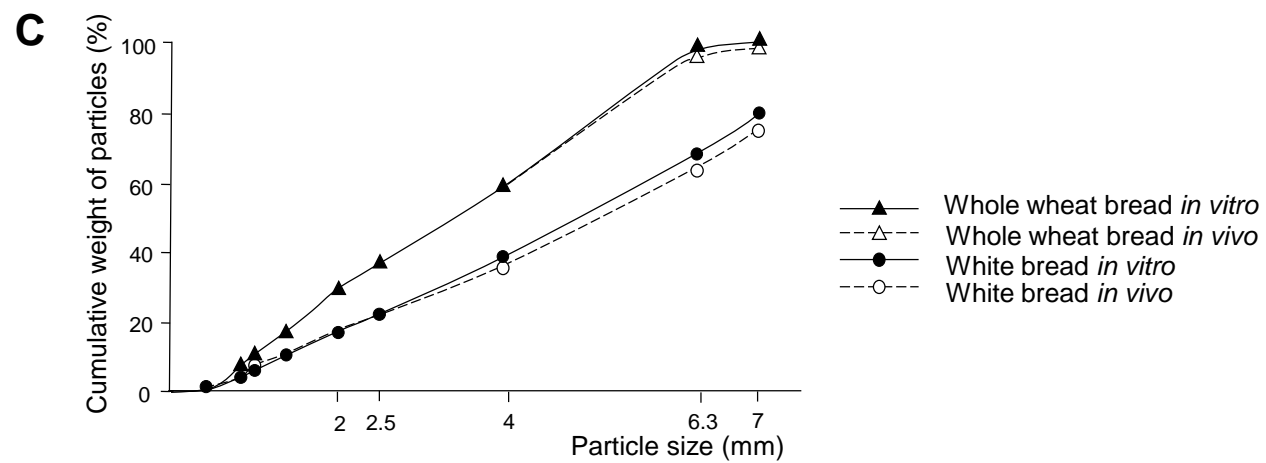
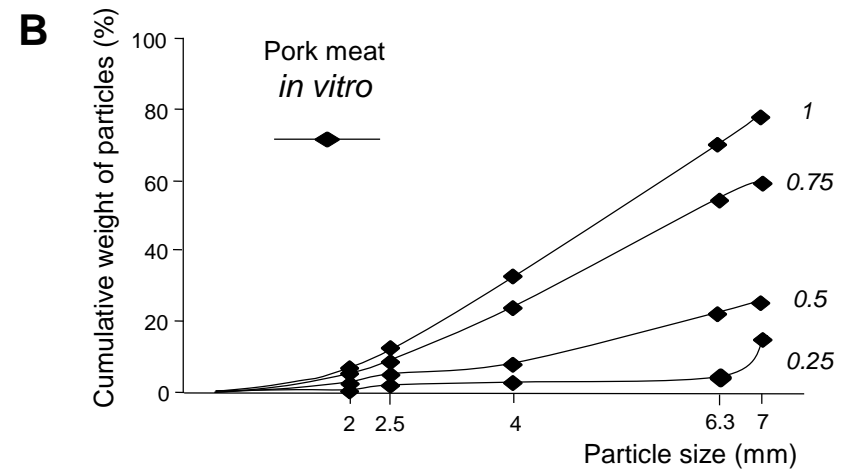
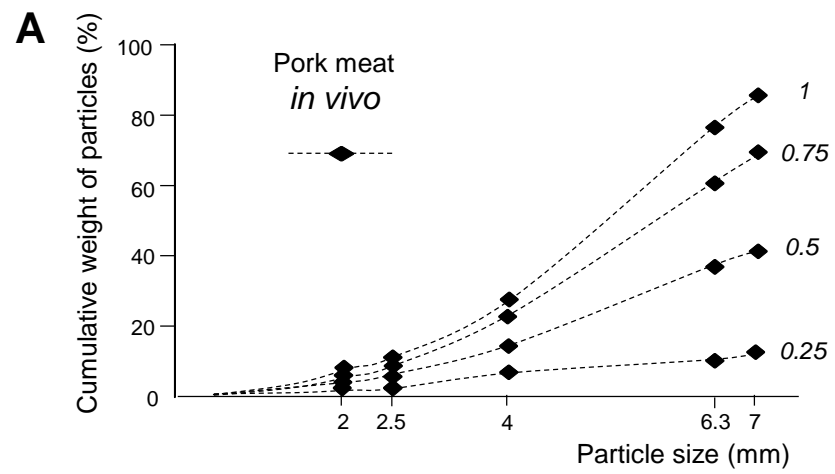


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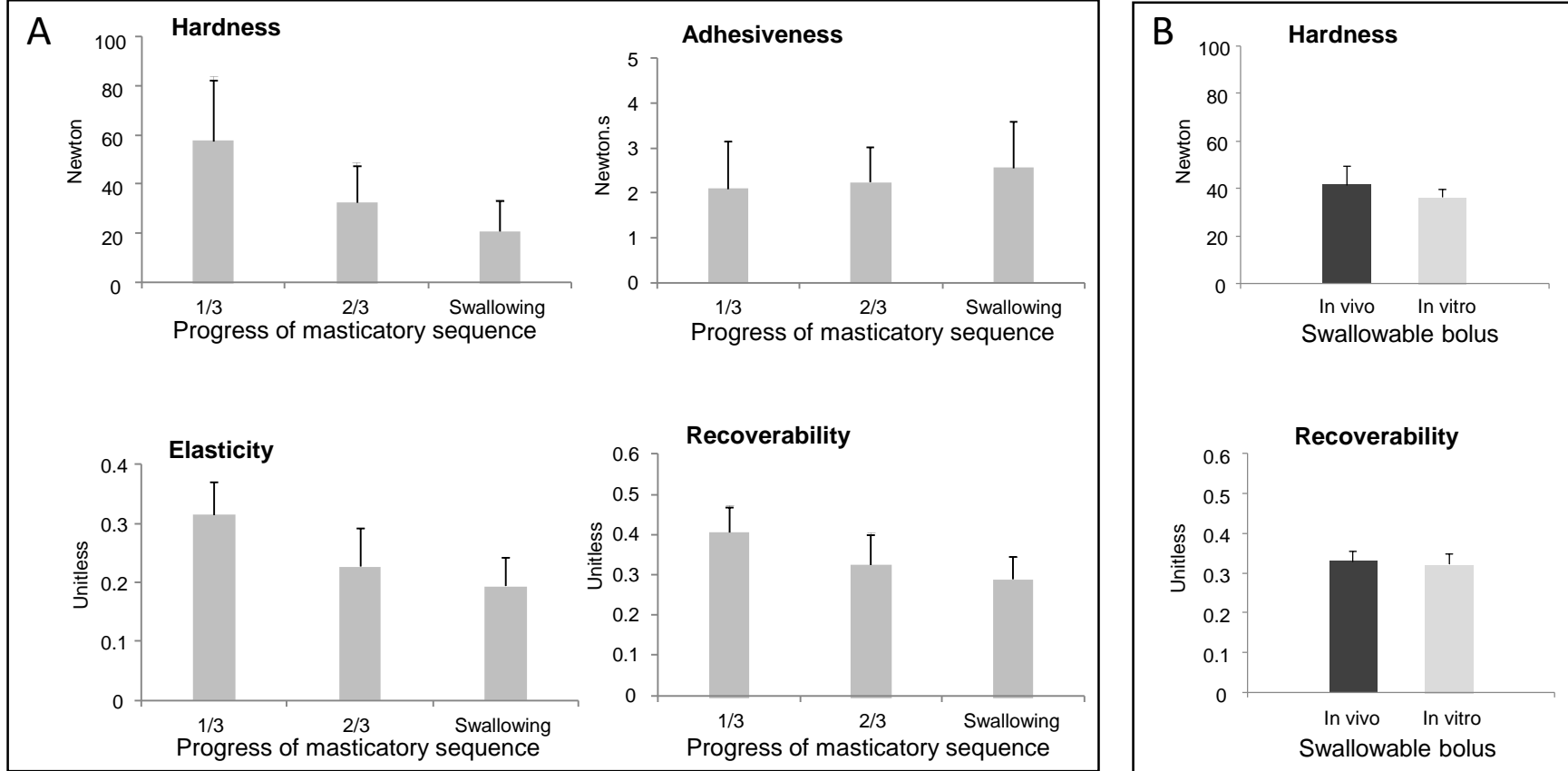


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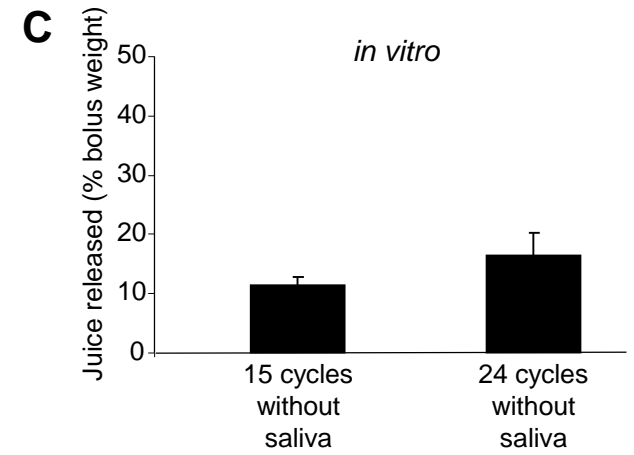
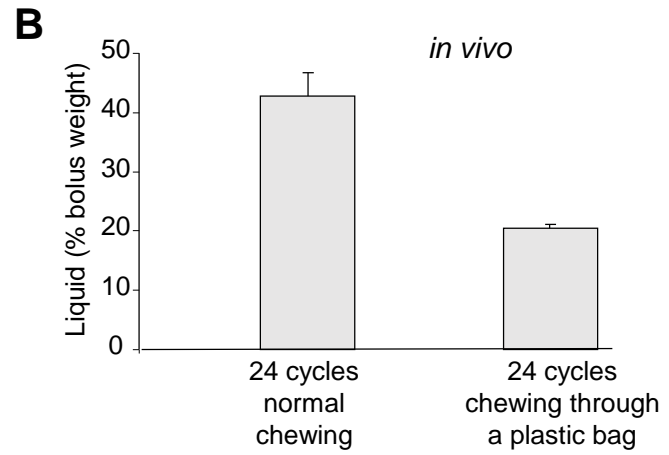
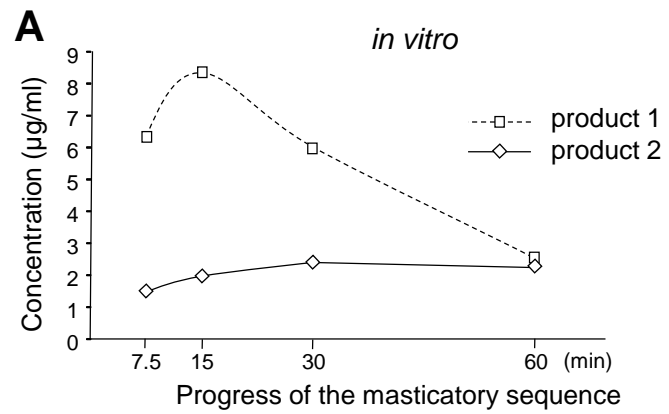


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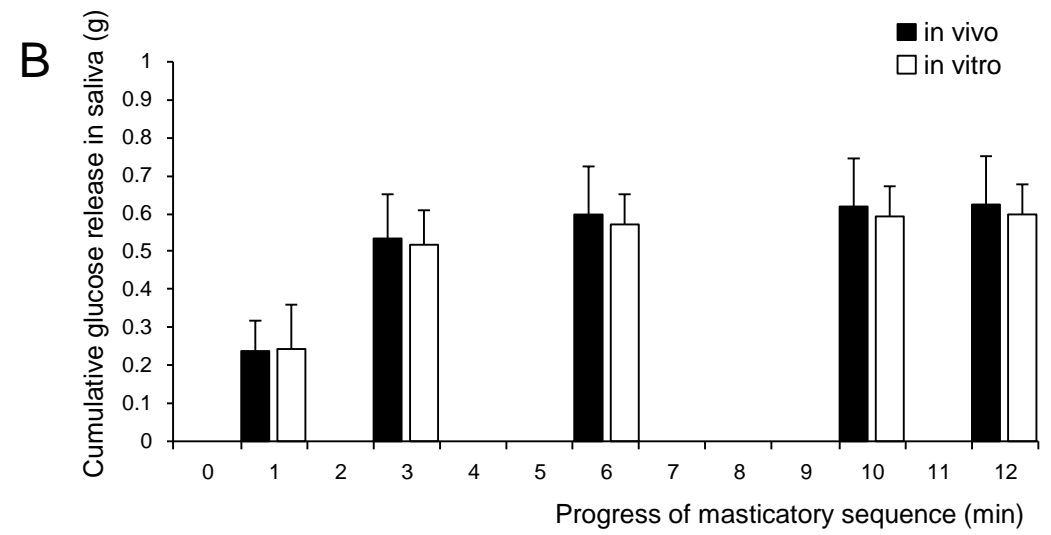
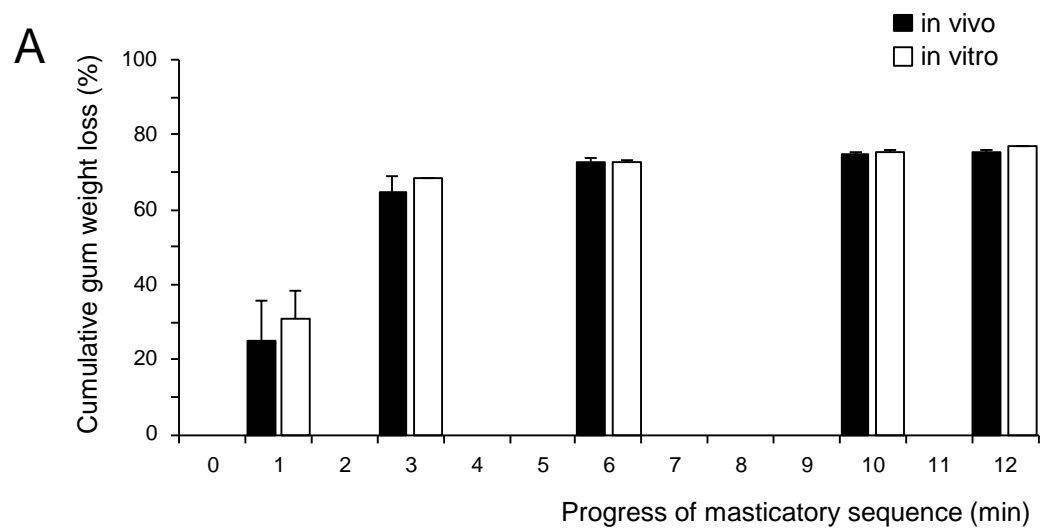


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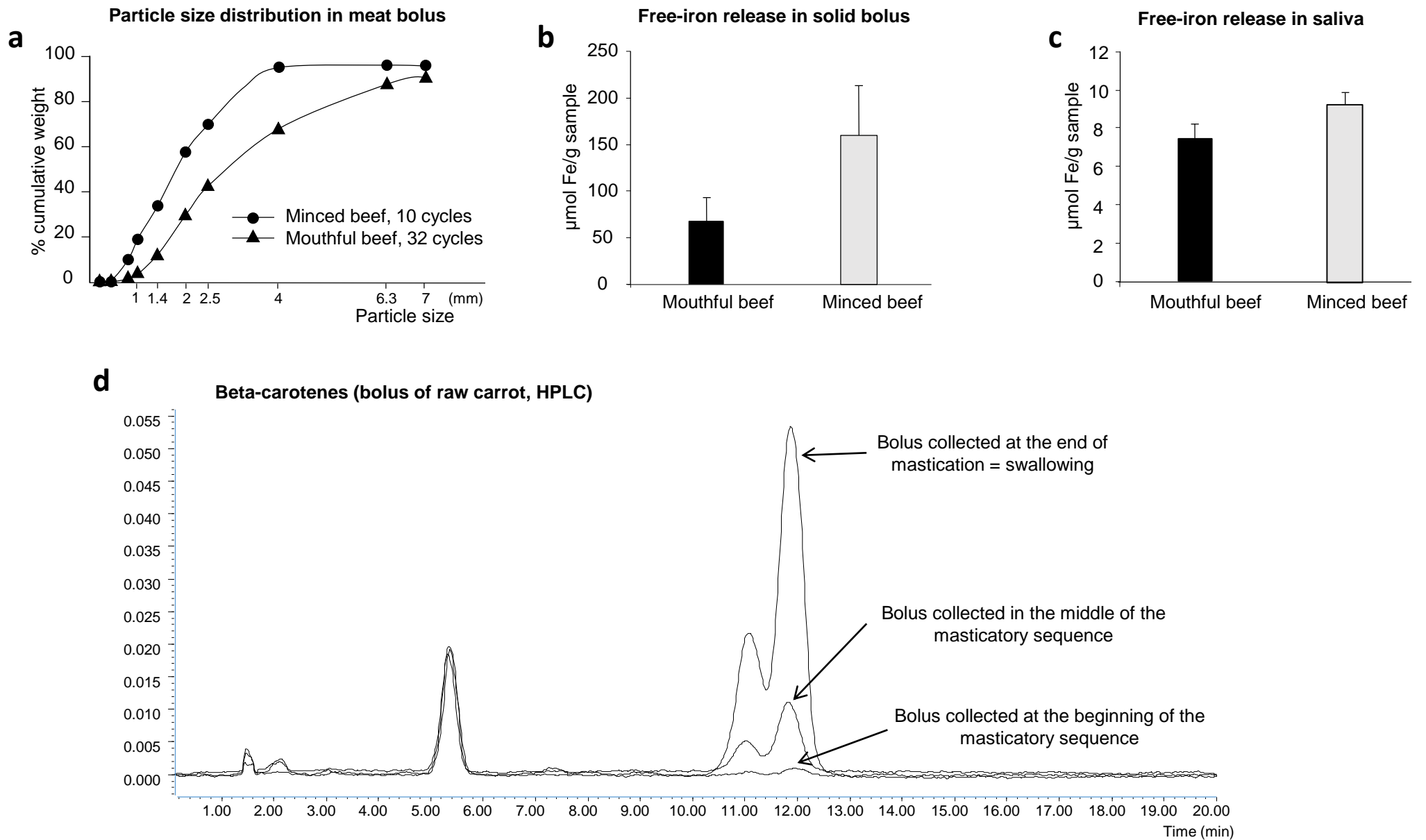


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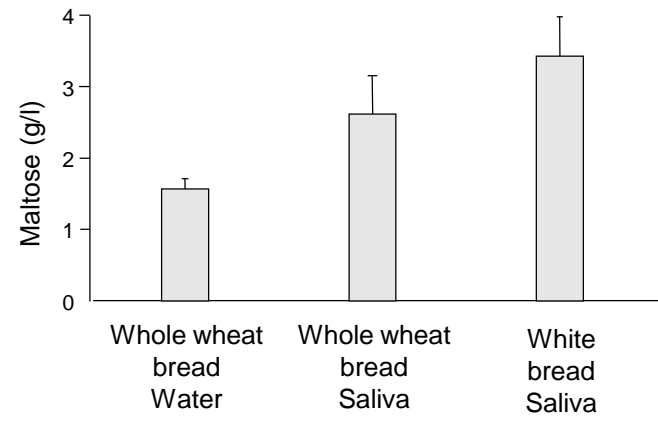
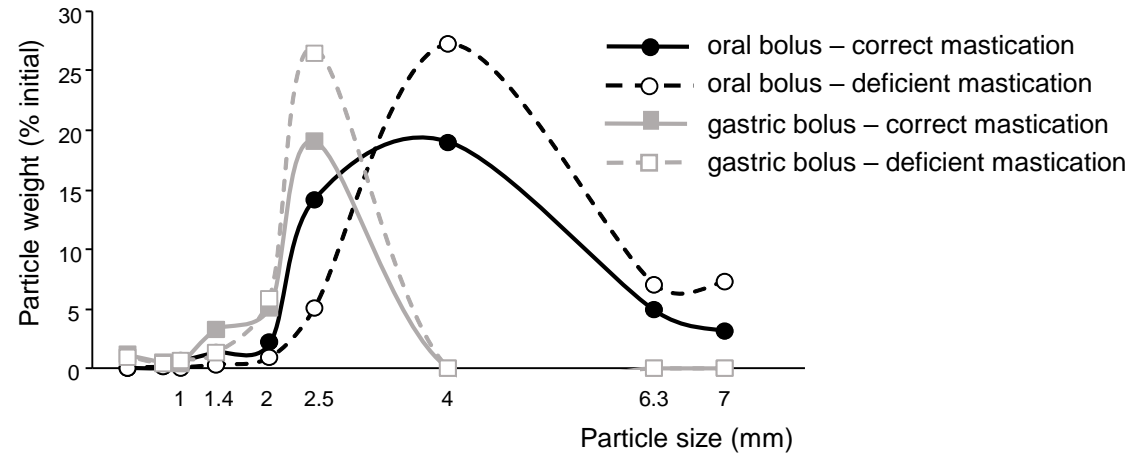


Figure 9

A



B

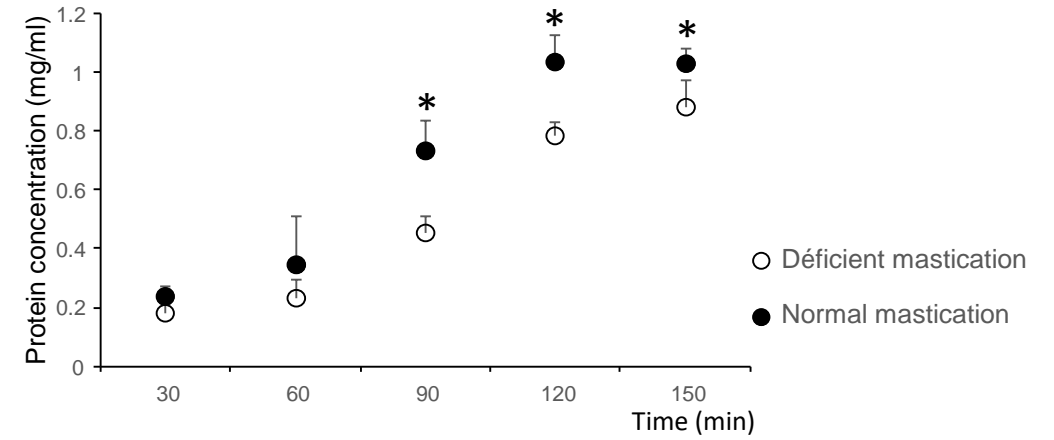


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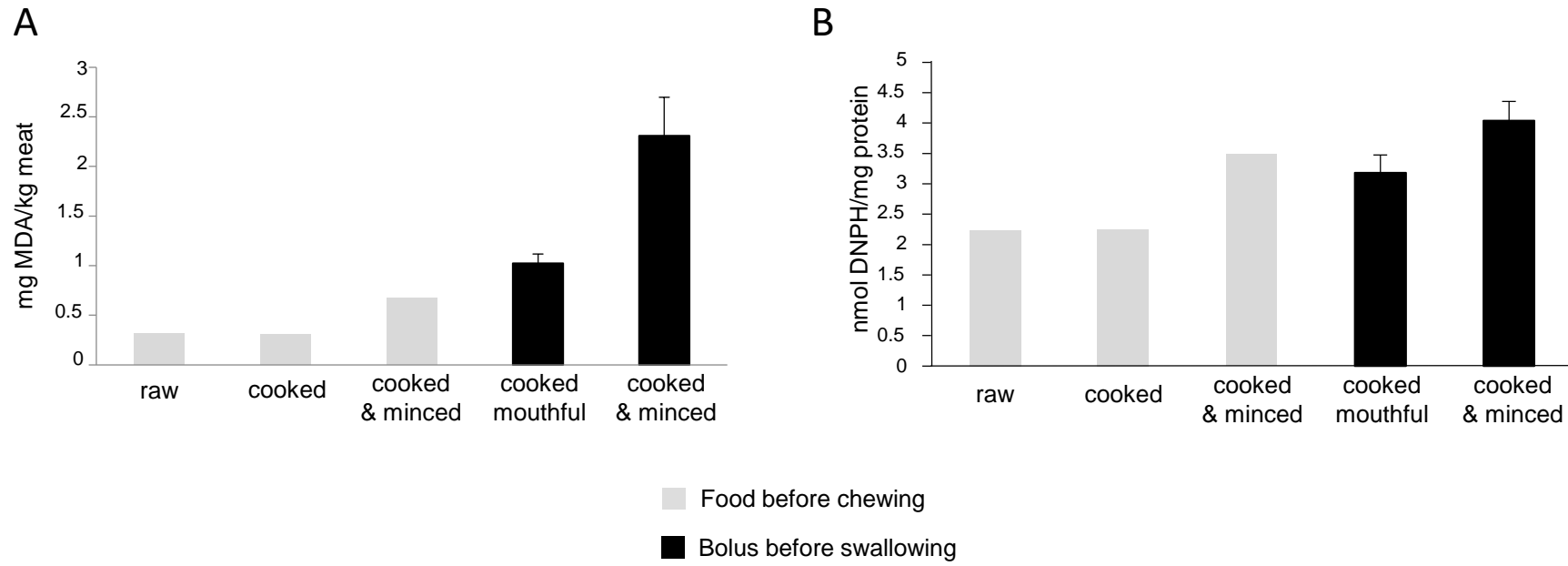


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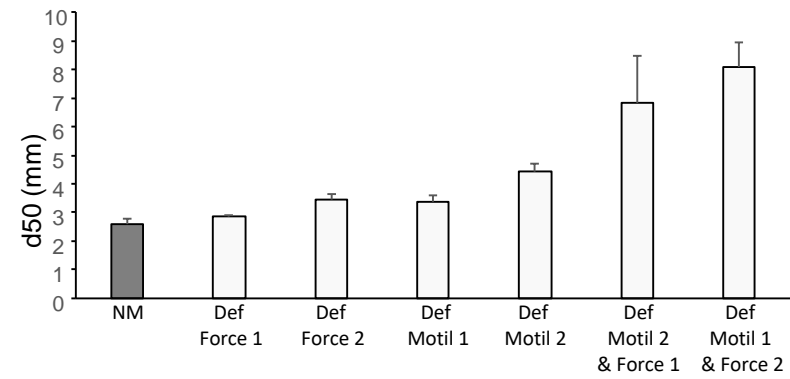


Figure 12

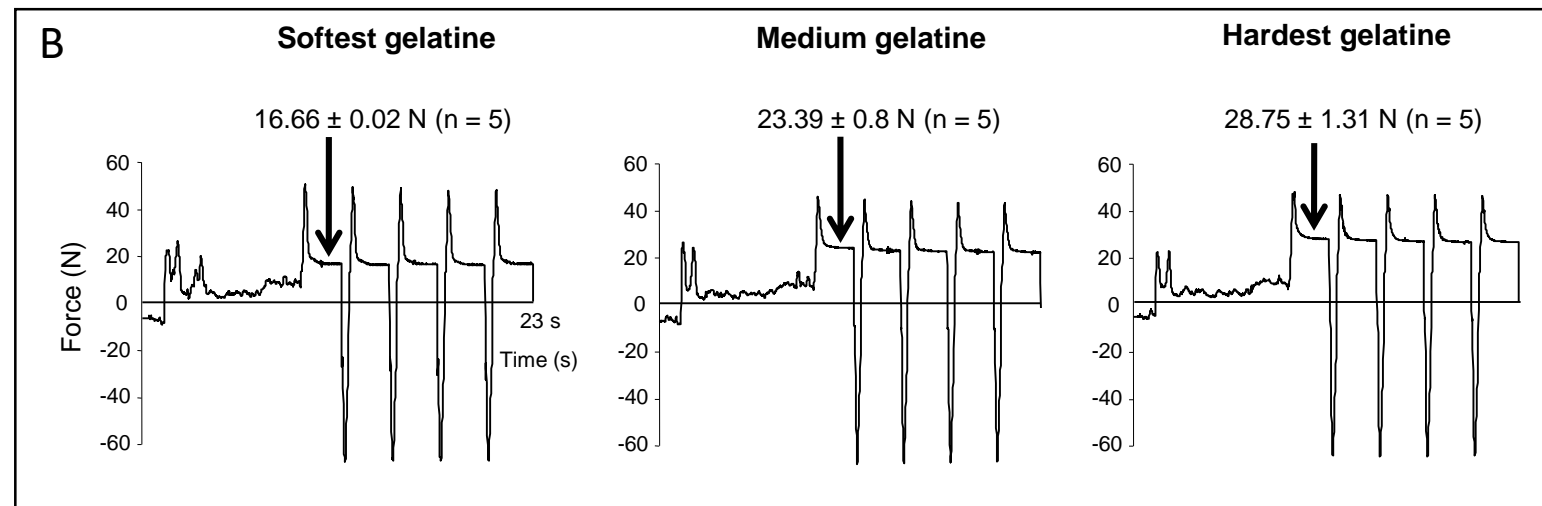
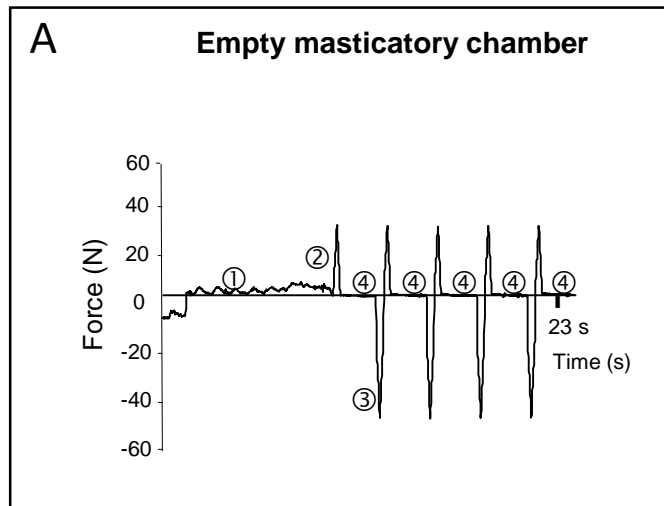
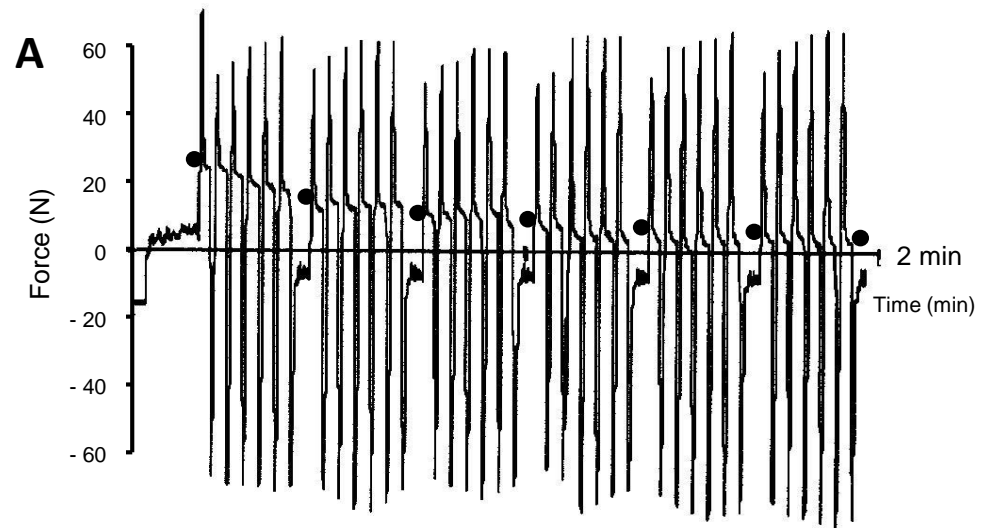
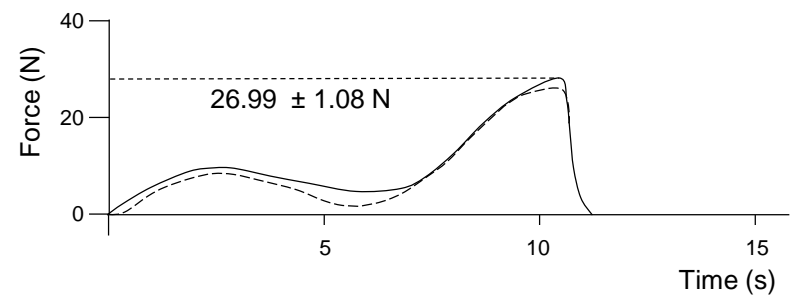


Figure 13

Chewing of banana sample in AM<sup>2</sup> in 36 cycles



**B** Instron: compression test; 2 banana samples



Five-bite compression of ripe and firm bananas samples in AM<sup>2</sup>

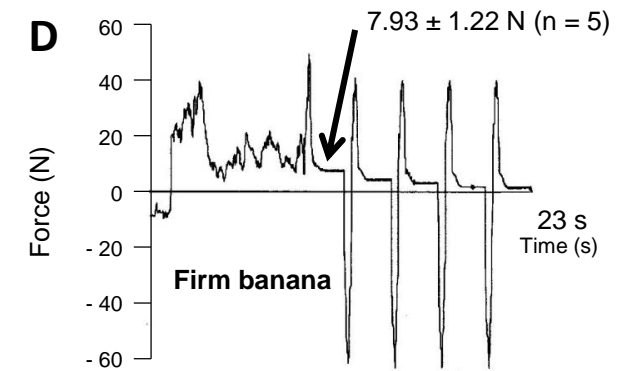
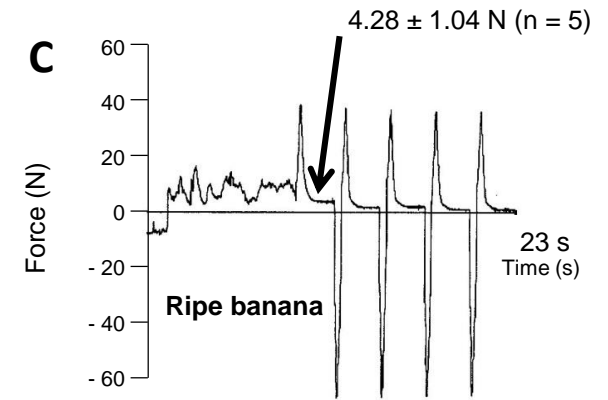


Figure 14

