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Additional Information

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2 **Gastrointestinal Conditions**

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Fat Digestibility in Meat Products: Influence of Food Structure and Gastrointestinal Conditions

Digestibility of macronutrients depends on the food matrix structure as well as on gastrointestinal conditions, especially in patients with exocrine pancreatic insufficiency. In this situation, an oral enzyme supplementation that promotes nutrient hydrolysis is needed. In this context, in the present study, a static in vitro digestion model was used to assess the lipid digestibility of different meat products (processed and fresh), different intestinal conditions of pH (6 or 7), bile concentration (1 or 10 mM), and doses of the enzyme supplement (1000 to 4000 lipase units/ g fat). Results showed that processed (unstructured) meats had better matrix degradation during digestion and reached higher values of lipolysis extents (total free fatty acids/ g fat) than the natural meat matrices with a statistically significant association ($p<0.001$). Regarding the intestinal medium, pH of 7 and bile concentration of 10 mM contribute to higher matrix degradation, and thus, to a higher lipolysis ($p<0.001$).

Keywords: in vitro digestion, lipolysis, nutrition, pancreatic insufficiency, food matrix

Introduction

Nowadays meat products are overconsumed in the western societies, both in young and adult populations, and the consumption exceeds the recommended daily intake (Huybrechts et al, 2010, Moreira et al, 2010, Pérez-Rodrigo et al. 2015, Roccaldo et al. 2014). Processed meats are the preferred choice over the fresh varieties because processed meat products are cheaper and easier to prepare. Moreover, the fast-food type restaurants, which offer mainly the varieties of meat that are already prepared and processed, are a frequent meal choice (Bhutani, Schoeller et al. 2018, Close et al. 2016, French et al. 2001).

Meat is an important source of protein and vitamins and its composition also contains fat and water (Pereira and Vicente 2013). Different meats and cuts are composed of distinctive types of muscle fibres and they contain varying amounts of connective tissues. This implies that the quality and structure of them are very diverse (Greenfield et al. 2009). In addition, the processing method of meat, ~~as well as~~

60   including the cooking technique, may also affect the final structure resulting in a wide

61   variety of food matrices in this food group (Bax et al.2012, Luo et al. 2018).

62   Concerning the fat fraction and quality of the meat, they depend on the area of the meat

63   portion as well as the origin of the animal.

64   While several studies assessing proteolysis in meat products have been

65   published in the recent years, scarce literature is available regarding the study of

66   lipolysis. Given the direct effect of dietary fat intake on the development of diet-based

67   diseases (de Souza et al. 2015), it is important to unveil the digestion fate of this

68   nutrient when it comes from a food choice as popular as meat.

69   Processed meats normally have a higher fat content than non-processed

70   varieties, as they are made of the animal parts that are less consumed, have less sensory

71   quality and a lower consumer acceptance. However, apart from the fat content, the

72   structure also could influence digestibility of this nutrient. Concretely, in natural

73   structure of meat, fat molecules are embedded in muscle fibres while processed meats

74   have an unstructured mixture of fat, protein and water, and in some cases, carbohydrates

75   are also added (Guo et al. 2017). Therefore, in order to address the study of lipid

76   digestibility in meat, the influence of the meat structure should be taken into account.

77   Lipid digestion is a complex process, which takes place in the duodenal region

78   of the small intestine, which implies a previous micellization in order to provide the

79   enzyme access allowing lipid hydrolysis. During this process, the intestinal pH and the

80   presence of bile salts are crucial to improve the enzymatic hydrolysis (Ryan et al. 2008,

81   Whitcomb et al. 2010). The intestinal pH is directly related to the enzyme activity and

82   efficiency. Moreover, the bile salts act as emulsifying agents leading to the

83   micellization of fat and providing a larger surface area on the fat globule for the enzyme

84   to adsorb. However, the alteration of these factors could compromise the lipid

85   digestibility in some individuals who suffer from exocrine pancreatic insufficiency

86   (EPI), mainly present in patients with Cystic Fibrosis. The obstruction of the pancreatic

87   duct in EPI produces an insufficient secretion of pancreatic juice, which contains the

88   digestive enzymes. Moreover, the secretion of sodium bicarbonate ($NaHCO_3$) is also

89   reduced, causing a drop in the intestinal pH and the consequent reduction of the

90   pancreatic enzymes activity. This situation leads to the mal-digestion and mal-

91   absorption of nutrients (Layer and Keller 2003, Naikwade et al. 2009). The treatment

92   for EPI consists in oral administration of pancreatic enzyme supplements, containing

93   proteases and mainly lipase (Armand et al. 2011). The current guidelines for nutritional

management in Cystic Fibrosis recommend a dose of the enzymatic supplement of 2000 – 4000 LU/ g fat, with a very low degree of evidence (Turck et al. 2016). The modulation of both intestinal pH and bile salts concentration in the intestinal digestion medium are thus of upmost relevance in the process of lipolysis.

In vitro studies are a useful tool that allows studying the structural changes, the digestibility and release of food components under simulated gastrointestinal conditions. In this context, the goal of the present study was to evaluate the influence of gastrointestinal conditions associated with EPI (intestinal pH, bile salts concentration and the dose of the enzyme supplement) as well as the effect of the food matrix on lipid digestibility in different meat products.

Materials and methods

Materials

The different meat products (hamburger (just the meat patty, 100% minced pork meat), sausage, luncheon ham, cooked ham, cured ham, pate, chicken drum, pork loin and beef steak were obtained from a local supermarket in Valencia (Spain).

For the preparation of the simulated digestive fluids (**Table 1**) the following chemicals were needed: human α – amylase (1000 – 3000 U/ mg protein) (CAS: 9001-19-8), pepsin from porcine gastric mucosa (≥2500 U / g protein) (CAS: 9001-75-6), bovine bile extract (CAS: 8008-63-7), KCl, KH_2PO_4, $NaHCO_3$, NaCl, $MgCl_2$ $(H2O)_6$, $(NH_4)_2CO_3$ and $CaCl_2$, all of them from Sigma-Aldrich Chemical Company (St Louis, MO, USA). NaOH (1 N) and HCl (1 N), were all acquired from AppliChem Panreac. Pancreatic enzymes supplements (Kreon 10,000 lipase units (LU)) were used to simulate in vitro digestion of an individual with EPI. Each capsule contains 150 mg of gastro-resistant microspheres containing porcine pancreatic enzyme equivalent to 10,000 lipase U., 8,000 amylase U., and 600 protease U. The specific lipase activity of the Kreon was usually measured before the experiments (Carriere et al. 2000) and the amount of supplement added to the gastric stage was adjusted always to have the corresponding LU/g fat according to the experimental design.

For the analytical determinations, Triton-X 100%, as well as the analytical standard of palmitic acid, were acquired from Sigma-Aldrich. Ethanol (96 % v/v for analysis) was acquired from AppliChem Panreac.

Sample preparation

128 Prior to the digestion process, hamburger, chicken drum, pork loin and beef steak were

129 placed in a microwavable plate and cooked in a household microwave oven (model

130 GW72N, Samsung) for 4 min at 600 W, 2450 MHz. For the rest of the matrices

131 (luncheon ham, pate, cured ham, cooked ham and sausage) a thermic treatment was not

132 necessary because they were ready to eat. After their preparation, all samples were in

133 vitro digested by using a static system.

134

135 *In vitro digestion process*

136 Meat samples were placed into falcon tubes (50 ml); the amount of samples to be

137 digested was weighted in order to have 0.35 g of fat in each tube. Fat, water and protein

138 content in all meat products was determined before digestion by the official methods

139 (AOAO, 2000).

140 The digestion proceeding used was based on the standardized static in vitro

141 digestion method for food published by Minekus et al. (2014) with some modifications

142 in order to allow for analysing EPI conditions. **Table 1** illustrates the amounts and

143 composition of the fluids required in each of the stages of the digestion process

144 (Minekus et al. 2014). The digestion fluids were prepared daily from stock solutions,

145 salivary (SSS), gastric (SGS) and intestinal (SIS) prepared according to Minekus et al.

146 (2014). The enzymatic activity was tested before each simulation following the protocol

147 proposed by Carriere et al., (2000)**.** The in vitro digestion process was performed as

148 follows:

149 Oral stage: Simulated salivary fluid (5 ml) (SSF; pH 8) at 37ºC, was added to

150 the meat sample in a 1:1 (w/v) ratio and properly homogenized with a kitchen blender

151 for 3 minutes (Vario Mixer, Ufesa 600 W). Salivary amylase was added into SSF to

152 reach a concentration in the saliva mixture of 75 U/ml.

153 Gastric stage: After the oral stage, simulated gastric fluid (SGF; pH 3) was

154 added to each tube containing the oral bolus (1:1 v/v). Pepsin was added into the SGF to

155 reach a concentration in the gastric mixture of 2000 U/ml. The pH of the mixtures was

156 adjusted with HCl (1 N) to pH 2.8 ± 0.1 and samples were flipped from top to bottom at

157 55 rpm for 2 hours using an Intell-Mixer RM-2 (Elmi Ltd, Riga, LV-1006, Latvia) and

158 incubated at 37 ºC in a chamber Selecta (JP Selecta SA, Barcelona). These mixing

159 conditions provided constant mechanical energy to induce the breakdown of the food

160 matrix during digestion. The pancreatic supplement was added in the gastric stage in

161 order to simulate swallowing the pill in case of EPI situations.

162   Intestinal stage: Following the gastric stage, simulated intestinal fluid (SIF; pH

163   7) was added in a 1:1 (v/v) proportion to each tube containing the gastric chime. The pH

164   of the mixtures was adjusted to pH 6.0 ± 0.1 or 7.0 ± 0.1, depending on the conditions

165   to be tested, with NaOH (1 N). Samples were then being agitated from top to bottom at

166   55 rpm for another 2 hours at 37 ℃. pH was monitored during the digestion process and

167   readjusted if necessary to keep it constant (González-Bacerio et al. 2010, Prazeres et al.

168   1994).

169

170   *Experimental design*

171   The experimental design for each type of product (hamburger, sausage, luncheon ham,

172   cooked ham, cured ham, pate, chicken drum, pork loin and beef steak) consisted of two

173   main sets of experiments. In the first, intestinal conditions were fixed at pH 6 and bile

174   salts concentration 1 mM, and different pancreatin supplement doses (0, 1000, 2000,

175   3000 and 4000 LU/g of lipid) were tested, in order to assess the influence of enzyme

176   concentration. In the second, the dose of enzymes was fixed at 2000 LU/ g of lipid, and

177   the study variables were different combinations of intestinal pH and bile concentration:

178   pH6 – 1 mM, pH 6 – 10 mM, pH 7 – 1 mM and pH 7 – 10 mM, in order to analyse the

179   impact of different intestinal scenarios on lipolysis, and matrix degradation. Of note, the

180   combination pH 6 – 1 mM would represent the most unfavourable condition in the

181   gastrointestinal tract in EPI (Clarke 2001, Gelfond et al. 2013, Harries et al. 1979,

182   Rovner et al. 2013, Vu et al. 2000), and the pH 7 – 10 mM would approach the standard

183   duodenal conditions of a healthy adult. All the experiments were conducted in triplicate.

184

185   *Analytical determinations*

186   *Matrix Degradation Index (MDI (%))*

187   Matrix Degradation Index (MDI) was determined in all samples after in vitro digestion.

188   This parameter represents the proportion of solids that were finely dispersed in the final

189   product of digestion. The total content of a digestion tube was centrifuged (4000 x g for

190   20 minutes, 4 ℃) and filtered by a metallic sieve (1.6 mm x 1.6 mm mesh) to separate

191   out large particles. The drained liquid was collected and used to determine the free fatty

192   acids. The solid particles were rinsed twice with 5 ml of appropriate juice to remove any

193   digested material. Blotting paper was placed around the metallic sieve for 10 minutes to

194   drain residual digestion juice. The solid meat particles were then transferred to an

195   aluminum dish and immediately weighed. The aluminum dish was put in a forced air

196 oven at 60 ℃ for 48 hours and weighed again to determine the mass of large solids. The

197 MDI, corresponding to the proportion of solids passing the metallic sieve, was

198 calculated according to Lamothe et al. (2012, 2014).

199 *Lipolysis extent (%)*

200 Drained juice from digested samples was diluted 100-fold with a solution made of 5.6%

201 Triton X-100 and 6% ethanol in water (Lamothe et al. 2012) **.** This solution was used to

202 solubilize the free fatty acids (FFA) and stop the lipase activity. Fatty acids release

203 during digestion was measured on the diluted samples using a free fatty acid

204 colorimetric assay kit (Roche Diagnostics, Indianapolis, IN, USA) and a

205 spectrophotometer (UV/vis, *Beckman Coulter*) (Lamothe et al. 2014). Palmitic acid

206 standard was used for quantitative determination of FFA. Total FFA was expressed as

207 mg of fatty acids released after a complete digestion per gram of initial fat present in

208 each meat product.

209

210 *Statistical analysis*

211 The variables included for the statistical analysis were the nutrient composition of

212 foods: water, protein and fat, starch; and the food matrix structure: natural food matrix

213 (protein fibres) or processed matrix (unstructured); the pancreatic enzyme supplement

214 concentration (enzyme dose); the intestinal conditions: pH and bile salts concentration;

215 and the matrix degradation index (MDI). The response variables were lipolysis extent

216 (total FFA) and the MDI.

217 Data were summarised using mean, standard deviation, median and 1st and 3rd

218 quartile in the case of continuous variables and with absolute ant relative frequencies in

219 the case of categorical variables.

220 Linear mixed regression models were performed to assess the effect of the food

221 matrix structure and other factors such as matrix degradation index were included as

222 covariates. Additionally, because observations of the same food are more likely to have

223 similar lipolysis extent due to their nutritional characteristics, the linear regression

224 models were extended with the "Food" variable as random effect with random intercept

225 to correct for the non-independence of the data.

226 All the analyses were performed by software R (version 3.4.2) using packages

227 betareg (version 3.1-0), lme4 (version 1.1-14) and NMF (version 0.20.6). A p-value

228 lower than 0.05 was considered statistically significant.

229

3. Results and discussion

Effect of the food structure on matrix degradation and fat digestibility

The degradation of the food matrix is the process by which the 3D structural conformation of a food is disrupted into smaller parts allowing for the release of the structural components, i.e. the nutrients (**Table 2**). It is facilitated by several mechanisms: mainly the mechanical forces produced along the gastrointestinal tract (chewing in the mouth, stomach walls agitation and small intestine peristaltic movements) and the enzymatic activity that contributes to the breakdown of nutrients conforming the matrix structure. Thus, when no enzymes were used in the in vitro digestion, significantly lower matrix degradation indexes were obtained (**Figure 1**) as compared to digestions conducted with pancreatic enzyme supplements. Generally, the enzymatic supplements increase of 1000 LU/ g of fat led to a minor increase in the MDI. In the case of the natural matrices (protein fibres) maximum MDI were between 50 and 75%, while in the unstructured matrices higher degradation extents were reached (>75%).

In terms of fat digestibility (total release of FFA), a similar tendency as in the MDI was shown for all the assessed meat products (**Figure 2**). In those matrices which preserve the protein fibres structure, the total FFA increased with the concentration of the enzymatic supplement up to 3000 LU/g fat, and it slightly decreased at 4000 LU/g fat, probably due to inactivation by aggregation. It has been described that when a high concentration of enzymes is not active in the reaction medium (e.g. because there is no substrate available) aggregation and inactivation occur. Furthermore, the release of fat particles from the protein fibres complex is a progressive and slow process, so this may lead to a low concentration of substrate at the beginning of the digestion. This may be the reason of the inactivation phenomenon (López-Gallego et al. 2005). In contrast, in unstructured matrices, the release of FFA was directly proportional to the concentration of enzymes. Pate and luncheon meat reached a total FFA released close to 600 mg/ g fat at enzyme dose of 4000 LU/ g fat. On the other hand, hamburger and sausage reached a lipolysis extent of 500 and 300 mg FFA/ g fat, respectively. In any case, the highest value of lipolysis was reached at enzyme dose of 3000 LU/ g fat.

The fact that MDI and total FFA released followed the same tendency was, indeed, statistically explained, finding significant associations between the type of matrix and the MDI, and between the MDI and the fat digestibility (**Figure 3**). The unstructured matrices allowed for higher MDI than the natural protein fibres, up to

264 30.6% more (p = 0.013, 95% CI [8.148, 30.612]); and the higher MDI were associated

265 with higher total FFA released, every 1% increase produced between 6.1 and 9.6 mg of

266 FFA more (p <0.001, 95% CI [6.128, 9.606]).

267 These relationships are backed up by previous literature providing a physical

268 explanation, as previously compiled by Guo et al. (2017). When fat molecules are

269 trapped in a solid food matrix, the structure of the surrounding food matrix is the

270 dominant factor controlling digestion. In order to make lipids available to the enzymes,

271 the degradation of the protein food matrix has to occur. In such systems, lipolysis is

272 thus conditioned by the rate and extent of proteolysis: as the protein structure is broken

273 down, the lipids are released from the matrix, and lipases can start hydrolysis

274 (Dickinson 2012). An example of this type of system was described by Dickinson

275 (2012), in which protein gels containing lipids were formulated with different amounts

276 of protein and forming different microstructures (hard and soft gels). The soft gel

277 presented an in vitro digestion behaviour similar to a liquid whey protein emulsion, but

278 the hard gel showed slower lipolysis rates and extents, as the gel structure was degraded

279 along digestion and allowed for fat droplets release from the matrix (Guo et al. 2017,

280 Guo et al. 2016).

281 Another example supporting our findings is the study of fat digestibility in

282 chewed almonds versus their isolated fat molecules, which lipolysis extents were 22 and

283 69% after one hour of in vitro digestion (Grundy et al. 2017). The study pointed at the

284 fact that cells remained largely intact after mastication and concluded that lipid

285 bioaccessibility in almonds was dependent on the structure and the cell walls

286 surrounding the oil droplets.

287 Finally, the role of the food structure in protein-lipid matrices has been also

288 assessed in two types of cheese (Fang et al. 2016). Cheddar cheese had larger fat

289 globules that made the structure less hard and more easily degraded. On the other hand,

290 the mozzarella had a denser fibrous protein matrix. In terms of digestibility, it was

291 higher in cheddar as fat globules were rapidly released and accessible to the enzymes,

292 while in mozzarella the fibrous structure prevented fat release and thus led to a lower

293 lipolysis extent.

294 In the present study, natural protein fibres matrix represents a robust structure in

295 which lipids are contained, and the unstructured matrices suppose a softer system in

296 which lipid and protein are not bonded or linked to each other. This structural fact

297 explains why the unstructured matrices had more MDI and consecutively a higher
298 amount of FFA release.

299 Apart from the food structure, the conditions of the digestive fluid and the
300 intestinal environment may also affect the behaviour of food digestion. These are
301 explored and explained in the coming section.

302

Effect of the intestinal conditions on matrix degradation and fat digestibility

304 In all the assessed intestinal conditions, the unstructured matrix meat products had
305 higher MDI than the structured, values ranging from 69 to 78.6% and 42 to 70%,
306 respectively (**Table 3**). The intestinal pH had a significant effect on the matrix
307 degradation index, as in all the assessed meat products it allowed for an increase
308 between 5 and 15% ($p < 0.01$). However, when comparing the effect of the bile salts
309 concentration at intestinal pH 6 or at pH 7, this showed a non-significant increase in the
310 matrix degradation index. Pate was an exception, in which MDI increased 7% at the 10
311 mM concentration, both in pH 6 and 7. In contrast, the role of bile was not significant in
312 proteolysis, but it was important on lipolysis due to the bile emulsify the lipids allowing
313 a higher surface area available for the lipases action.

314 Overall, in terms of fat digestibility, the release of total FFA was significantly
315 higher at pH 7 ($p < 0.001$, 95% CI [53.5, 168.9]) and at bile salts concentration of 10
316 mM ($p < 0.001$, 95% CI [90.2, 205.6]) (**Figure 4**). The 95% CI represent the extent to
317 which the pH 7 and bile 10 mM conditions produce an increase in the total FFA. This is
318 in accordance to previous studies conducted by our group (Asensio-Grau et al. 2018,
319 Peinado et al. 2018)

320 Focusing on the results when considering the food products and their type of
321 matrix, more concrete patterns could be depicted (**Table 4**). Digestions conducted at
322 intestinal pH 7, revealed that in the protein fibre matrices the higher concentration of
323 bile salts led to a significant increase in total FFA release of up to 300 mg / g fat, while
324 this effect of bile concentration showed a lower increase in the unstructured matrices
325 (50 mg/ g fat, except for luncheon ham whose increase was higher). In contrast, the
326 unstructured matrices were more influenced by the 10mM bile salts concentration in the
327 intestinal pH 6 scenario, which produced an increase of around 150 mg of FFA released
328 per gram of fat as compared to the use of 1mM bile. At this last pH 6 and 1mM
329 combination, the natural protein fibres matrices did not show a common pattern.
330 Although the 10 mM concentration allowed for a higher amount of FFA release in all

the meat products, the increase was up to different extents: the highest was in beef steak (400 mg/g fat) and the lowest in chicken drum (which showed no increase).

Thus, at intestinal pH 7 bile concentration plays a crucial role in natural matrices while it is not relevant in the unstructured matrices; and at pH 6, the higher bile concentration improves FFA release in unstructured matrices but shows a rather random effect in the natural ones. On the one hand, bile salts are bio-surfactant compounds that play a crucial role in lipid digestion. They adsorb onto lipid droplets and remove proteins, emulsifiers and free fatty acids (lipolysis products). This mechanism facilitates lipases' access and action (Maldonado-Valderrama et al. 2011, Harries et al. 1979). On the other hand, the intestinal pH environment determines enzymatic activity and also influences the isoelectric point of the protein that may be present at the lipid droplet interface causing isoelectric effects. This fact may change the protein to a cationic form allowing the bind to the anionic bile salts. Consequently, different meat matrices result in different systems during digestion in which proteins can interact with the bile salts either promoting or inhibiting the activity of pancreatic lipase (Bauer et al. 2005, Lowe 2002).

To sum up, unstructured matrices led to higher MDI than the structured ones; higher MDI led to higher lipolysis extents (%); and the intestinal pH 7 and the 10 mM bile salts concentration allowed for higher lipolysis extents. These associations, explained by means of linear regression models, are summarised in **Table 5**.

Overall, a main limitation has been identified when aiming at discussing the results. There are no studies conducted in meat products assessing lipolysis, and in contrast, all of them focus on proteolysis. Nonetheless, two papers related to this topic were found: one study assessing lipid digestibility of pork patties depending on the thermal treatment (Hur et al. 2014), and another compiling knowledge about factors affecting lipolysis in fish (Olsen and Ringø 1997). This scarcity has prevented the comparison of the results to previous literature. This is a point of special relevance: characterising the role of lipid digestion in meat products is of upmost necessity, as recently the consumption of red meat products and cold meat derivatives has been classified by the World Health Organisation as a major determinant in the development of cancer (McGuire 2016). In addition, processed meat products have been traditionally characterised by a high content of fat, mainly composed of saturated fatty acids. This type of fat is unequivocally associated to the development of nutrition related conditions such as high blood pressure and diseases like obesity (Chen et al. 2016).

365

Conclusion

In conclusion, fat digestibility in a wide range of meat products has been screened and characterised for the first time regarding their composition, structure and intestinal digestion conditions. The results reveal that lipolysis extent is dependent on the food matrix degradation, and that unstructured processed meats allow for higher amount of free fatty acids release than the natural protein fibrous matrices. In addition to the food structure, the bile salts concentration in the digestion and the pH in the intestinal digestion medium, contribute significantly to the fate of lipid digestion in meat products.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

AOAC. 2000. Official methods of analysis of AOAC International. (17th ed.). Gaithersberg, Maryland: Association of Official Chemists.

Armand, Fieker, A. P., & Philpott, J. (2011). Enzyme replacement therapy for pancreatic insufficiency: present and future. Clin. Exp. Gastroenteroll. 4: 55. https://doi.org/10.2147/CEG.S17634

Asensio-grau A, Peinado I, Heredia A, Andrés A. 2018. Effect of cooking methods and intestinal conditions on lipolysis , proteolysis and xanthophylls bioaccessibility of eggs. J. Funct Foods.46: 579–586. https://doi.org/10.1016/j.jff.2018.05.025

Bauer E, Jakob S, Mosenthin R. 2005. Principles of physiology of lipid digestion.

399   Asian-Australas J. Anim. Sci. 18: 282–295.

400   Bax ML, Aubry L, Ferreira C, Daudin JD, Gatellier P, Rémond D, Santé-Lhoutellier V.
401   2012. Cooking temperature is a key determinant of in vitro meat protein digestion
402   rate: Investigation of underlying mechanisms. J. Agr. Food Chem. 60: 2569–2576.
403   https://doi.org/10.1021/jf205280y

404   Bhutani S, Schoeller DA, Walsh MC, McWilliams C. 2018. Frequency of Eating Out at
405   Both Fast-Food and Sit-Down Restaurants Was Associated With High Body Mass
406   Index in Non-Large Metropolitan Communities in Midwest. Am. J. Health Pr.
407   32(1), 75–83. https://doi.org/10.1177/0890117116660772

408   Carriere F, Renou C, Lopez V, De Caro J, Ferrato F, Lengsfeld H, Verger, R. 2000. The
409   specific activities of human digestive lipases measured from the in vivo and in
410   vitro lipolysis of test meals. Gastroenterology. 119: 949–960.
411   https://doi.org/10.1053/gast.2000.18140

412   Chen M, Li Y, Sun Q, Pan A, Manson JE, Rexrode KM, Hu FB. 2016. Dairy fat and
413   risk of cardiovascular disease in 3 cohorts of US adults. Am. Journal of Clin Nutr.
414   104. 1209–1217. https://doi.org/10.3945/ajcn.116.134460

415   Clarke LL, Stien X, Walker NM. 2001. Intestinal bicarbonate secretion in cystic fibrosis
416   mice. JOP : J. Pancreas. 2: 263–267.

417   Close MA, Lytle LA, Viera AJ. 2016. Is frequency of fast food and sit-down restaurant
418   eating occasions differentially associated with less healthful eating habits? Prev.
419   Med. 4, 574–577. https://doi.org/10.1016/j.pmedr.2016.10.011

420   De Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Anand SS. 2015.
421   Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality,
422   cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of
423   observational studies. Bmj, h3978. https://doi.org/10.1136/bmj.h3978

424   Dickinson E. 2012. Emulsion gels: The structuring of soft solids with protein-stabilized
425   oil droplets. Food Hydrocolloids. 28: 224–241.
426   https://doi.org/10.1016/j.foodhyd.2011.12.017

427   Fang X, Rioux LE, Labrie S, Turgeon SL. 2016. Disintegration and nutrients release
428   from cheese with different textural properties during in vitro digestion. Food
429   Research International, 88, 276–283. https://doi.org/10.1016/j.foodres.2016.04.008

430   French SA, Story M, Neumark-Sztainer D, Fulkerson JA, Hannan P. 2001. Fast food
431   restaurant use among adolescents: Associations with nutrient intake, food choices
432   and behavioral and psychosocial variables. Int. J. Obes. 25: 1823–1833.

433   https://doi.org/10.1038/sj.ijo.0801820

434   Gelfond D, Ma C, Semler J, Borowitz D. 2013. Intestinal ph and gastrointestinal transit

435   profiles in cystic fibrosis patients measured by wireless motility capsule. Dig. Dis.

436   Sci. 58: 2275–2281. https://doi.org/10.1007/s10620-012-2209-1

437   González-Bacerio J, Hernández JR, Martínez ADM. 2010. Las lipasas: enzimas con

438   potencial para el desarrollo de biocatalizadores inmovilizados por adsorción

439   interfacial. Rev. Colomb. Bio. 12: 124–140.

440   Greenfield H, Arcot J, Barnes JA, Cunningham J, Adorno P, Stobaus T, Muller WJ.

441   2009. Nutrient composition of Australian retail pork cuts 2005/2006. Food Chem.

442   117: 721–730. https://doi.org/10.1016/j.foodchem.2009.04.048

443   Grundy MML, Quint J, Rieder A, Ballance S, Dreiss CA, Cross KL, Wilde PJ. 2017.

444   The impact of oat structure and β-glucan on in vitro lipid digestion. J. Funct.

445   Foods. 38, 378–388. https://doi.org/10.1016/j.jff.2017.09.011

446   Guo Q, Ye A, Bellissimo N, Singh H, Rousseau D. 2017. Modulating fat digestion

447   through food structure design. Prog. Lipid Res. 68: 109–118.

448   https://doi.org/10.1016/j.plipres.2017.10.001

449   Guo Q, Ye A, Lad M, Dalgleish D, Singh H. 2016. Impact of colloidal structure of

450   gastric digesta on in-vitro intestinal digestion of whey protein emulsion gels. Food

451   Hydrocolloids. 54: 255–265. https://doi.org/10.1016/j.foodhyd.2015.10.006

452   Hur SJ, Lee SY, Moon SS, Lee SJ. 2014. In vitro effects of cooking methods on

453   digestibility of lipids and formation of cholesterol oxidation products in pork.

454   Korean J. Food Sci. An. 34: 280-286.

455   Huybrechts I, Maes L, Vereecken C, De Keyzer W, De Bacquer D, De Backer G, De

456   Henauw S. 2010. High dietary supplement intakes among Flemish preschoolers.

457   Appetite, 54(2), 340–345. https://doi.org/10.1016/j.appet.2009.12.012

458   Lamothe S, Azimy N, Bazinet L. 2014. Function Interaction of green tea polyphenols

459   with dairy matrices in a simulated gastrointestinal environment. Food Func.

460   5:2621–2631. https://doi.org/10.1039/C4FO00203B

461   Lamothe S, Corbeil MM, Turgeon SL, Britten M. 2012. Influence of cheese matrix on

462   lipid digestion in a simulated gastro-intestinal environment. Food Func. 3: 724–31.

463   https://doi.org/10.1039/c2fo10256k

464   Layer P, Keller J. 2003. Lipase Supplementation Therapy: Standards, Alternatives, and

465   Perspectives. Pancreas. 26: 1–7. https://doi.org/10.1097/00006676-200301000-

466   00001

467 López-Gallego F, Betancor L, Hidalgo A, Alonso N, Fernández-Lafuente R, Guisán JM.

468   2005. Co-aggregation of enzymes and polyethyleneimine: A simple method to

469   prepare stable and immobilized derivatives of glutaryl acylase.

470   Biomacromolecules. 6: 1839–1842. https://doi.org/10.1021/bm050088e

471 Lowe ME. 2002. The triglyceride lipases of the pancreas. J. Lipid Res. 43: 2007–2016.

472 Luo J, Taylor C, Nebl T, Ng K, Bennett LE. 2018. Effects of macro-nutrient, micro-

473   nutrient composition and cooking conditions on in vitro digestibility of meat and

474   aquatic dietary proteins. Food Chem. 254: 292–301.

475   https://doi.org/10.1016/j.foodchem.2018.01.164

476 Maldonado-Valderrama J, Wilde P, MacIerzanka A, MacKie A. 2011. The role of bile

477   salts in digestion. Adv. Colloid Interface Sci. 165: 36–46.

478   https://doi.org/10.1016/j.cis.2010.12.002

479 Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Brodkorb A. 2014.

480   A standardised static in vitro digestion method suitable for food-an international

481   consensus. Food Func. 5: 1113–1124. https://doi.org/10.1039/c3fo60702j

482 Moreira P, Santos S, Padrão P, Cordeiro T, Bessa M, Valente H, Moreira A. 2010. Food

483   patterns according to Sociodemographics, physical activity, sleeping and obesity in

484   Portuguese children. Int. J. Environ. Res. 7: 1121–1138.

485   https://doi.org/10.3390/ijerph7031121

486 Naikwade SR, Meshram RN, Bajaj AN. 2009. Preparation and In Vivo Efficacy Study

487   of Pancreatin Microparticles as an Enzyme Replacement Therapy for Pancreatitis.

488   Drug Dev. Ind. Pharm. 35: 417–432. https://doi.org/10.1080/03639040802422104

489 Harries JT, Muller DP, McCollum JP, Lipson A, Roma E, Norman AP. 1979. Intestinal

490   bile salts in cystic fibrosis: studies in the patient and experimental animal. Arch.

491   Dis. Child. 54: 19-24.

492 Olsen RE and Ringø E. 1997. Lipid digestibility in fish: a review. Recent Res. Dev.

493   Lipid Res.1:199-265.

494 Peinado I, Larrea V, Heredia A, Andrés A. 2018. Lipolysis kinetics of milk-fat

495   catalyzed by an enzymatic supplement under simulated gastrointestinal conditions.

496   Food Biosci. 23: 1–8. https://doi.org/10.1016/j.fbio.2018.02.011

497 Pereira PM. de CC, Vicente A. F. dos R. B. 2013. Meat nutritional composition and

498   nutritive role in the human diet. Meat Sci. 93: 586–592.

499   https://doi.org/10.1016/j.meatsci.2012.09.018

500 Pérez-Rodrigo C, Gil Á, González-Gross M, Ortega RM., Serra-Majem L, Varela-

501   Moreiras G, Aranceta-Bartrina J. 2015. Clustering of dietary patterns, lifestyles,

502   and overweight among Spanish children and adolescents in the ANIBES study.

503   Nutrients, 8(1), 1–17. https://doi.org/10.3390/nu8010011

504 Prazeres DMF, Garcia FAP, Cabral MS. 1994. Bioprocess Engineering Continuous

505   lipolysis in a reversed micellar membrane bioreactor, 10, 21–27.

506 Roccaldo R, Censi L, D'Addezio L, Toti E, Martone D, D'Addesa D, Cernigliaro A.

507   2014. Adherence to the Mediterranean diet in Italian school children (The ZOOM8

508   Study). Int- J. Food Sci. Nutr. 65: 621–628.

509   https://doi.org/10.3109/09637486.2013.873887

510 Rovner AJ, Schall JI, Mondick JT, Zhuang H, Mascarenhas MR. 2013. Delayed small

511   bowel transit in children with cystic fibrosis and pancreatic insufficiency. J.

512   Pediatr. Gastroenterol. Nutr. 57(1), 81–84.

513   https://doi.org/10.1097/MPG.0b013e318290d112

514 Ryan L, O'Connell O, O'Sullivan L, Aherne SA, O'Brien NM. 2008. Micellarisation of

515   Carotenoids from Raw and Cooked Vegetables. Plant Food Hum. Nutr. 63: 127–

516   133. https://doi.org/10.1007/s11130-008-0081-0

517 Turck D, Braegger CP, Colombo C, Declercq D, Morton A, Pancheva R, Wilschanski

518   M. 2016. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants ,

519   children , and adults with cystic fi brosis. Clin. Nutr. 35: 557–577.

520   https://doi.org/10.1016/j.clnu.2016.03.004

521 Vu MK, Vecht J, Eddes EH, Biemond I, Lamers CB, Masclee AA. 2000.

522   Antroduodenal motility in chronic pancreatitis: are abnormalities related to

523   exocrine insufficiency? NAL AND LIVER PHYSIOLOGY Am. J. Physiol.-

524   Gastroint. Liver Physiol. 278: 458–466.

525 Whitcomb DC, Lehman GA, Vasileva G, Malecka-Panas E, Gubergrits N, Shen Y,

526   Caras S. 2010. Pancrelipase delayed-release capsules (CREON) for exocrine

527   pancreatic insufficiency due to chronic pancreatitis or pancreatic surgery: A

528   double-blind randomized trial. Am. J. Gastroenterol. 105: 2276–2286.

529   https://doi.org/10.1038/ajg.2010.201

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TABLES

533

534   **Table 1.** Composition of simulated digestion fluids.

Constituent	SSF	SGF	SIF
	mmol/ L	mmol/ L	mmol/ L
KCl	15.1	6.9	6.8
KH_2PO_4	3.7	0.9	0.8
$NaHCO_3$	13.6	25	85
NaCl	-	47.2	38.4
$MgCl_2(H_2O)_6$	0.15	0.1	0.33
$(NH_4)_2CO_3$	0.06	0.5	-
$CaCl_2$	1.5	0.15	0.6

535 The addition of pepsin, Ca^{2+} solution and water will result in the correct
536 electrolyte concentration in the final digestion mixture.
537 SSF: Simulated Salival Fluid; SGF: Simulated Gastric Fluid; SIF:
538 Simulated Intestinal Fluid.

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558 **Table 2.** Characterization of water, fat and protein content in the different meat matrices
559 expressed as g/ g of product.

	Water content (g/ g product)	Fat content (g/ g product)	Protein content (g/ g product)
Hamburger	0.51 ± 0.03	0.24 ± 0.04	0.21 ± 0.02
Sausage	0.683 ± 0.002	0.107 ± 0.003	0.18 ± 0.02
Luncheon ham	0.653 ± 0.002	0.120 ± 0.006	0.11 ± 0.003
Pate	0.606 ± 0.004	0.30 ± 0.09	0.090 ± 0.002
Cured ham	0.529 ± 0.03	0.1 ± 0.02	0.32 ± 0.05
Cooked ham	0.756 ± 0.004	0.025 ± 0.03	0.199 ± 0.006
Chicken drum	0.649 ± 0.012	0.12 ± 0.03	0.201 ± 0.004
Pork loin	0.543 ± 0.026	0.135 ± 0.06	0.29 ± 0.04
Beef steak	0.56 ± 0.014	0.10 ± 0.03	0.30 ± 0.03

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580   **Table 3.** Matrix Degradation Index (%) obtained for the different meat matrices
581   (hamburger, sausage, luncheon ham, pate, cured ham, cooked ham, chicken drum, pork
582   loin and beef steak) after the in vitro digestion process using a fixed enzyme dose (2000
583   LU/ g fat) and different duodenal conditions of pH and Bile concentration.

	pH 6 - 1 mM	pH 6 – 10 mM	pH 7 – 1 mM	pH 7 – 10 mM
MDI (%)				
Hamburger	69 ± 2	69.06 ± 1.12	83 ± 4	82.1 ± 0.9
Sausage	82 ± 6	79 ± 3	94 ± 2	96.6 ± 1.2
Luncheon ham	86 ± 3	87.8 ± 0.5	90.38 ± 0.13	90.2 ± 1.4
Pate	78.6 ± 0.6	85.13 ± 1.14	81.2 ± 0.2	88.957 ± 1.014
Cured ham	70 ± 5	73.6 ± 0.2	67 ± 5	78 ± 6
Cooked ham	55.7 ± 0.3	55.13 ± 0.13	59.1 ± 1.2	63.3 ± 0.6
Chicken drum	63.57 ± 1.06	66 ± 3	69 ± 4	74 ± 2
Pork loin	52 ± 9	52 ± 6	68 ± 4	68.73 ± 0.04
Beef steak	42 ± 2	45 ± 3	51 ± 3	56 ± 2

Table 4. Total FFA (mg/ g fat) obtained for the different meat matrices (hamburger, sausage, luncheon ham, pate, cured ham, cooked ham, chicken drum, pork loin and beef steak) after the in vitro digestion process using a fixed enzyme dose (2000 LU/ g fat) and different duodenal conditions of pH and Bile concentration.

	pH 6 - 1 mM	pH 6 – 10 mM	pH 7 – 1 mM	pH 7 – 10 mM
Total FFA (mg/ g fat)				
Hamburger	253 ± 13	376 ± 58	501 ± 149	554 ± 115
Sausage	525 ± 22	667 ± 26	415 ± 44	490 ± 67
Luncheon ham	498 ± 18	619 ± 3	705 ± 1	902 ± 77
Pate	613 ± 153	710 ± 219	680 ± 41	700 ± 147
Cured ham	344 ± 80	467 ± 66	409 ± 85	600 ± 3
Cooked ham	298 ± 29	365 ± 162	416 ± 131	600 ± 177
Chicken drum	248 ± 8	249 ± 6	142 ± 109	408 ± 142
Pork loin	226 ± 8	614 ± 67	556 ± 104	653 ± 29
Beef steak	298 ± 4	783 ± 10	675 ± 199	860 ± 272

592 **Table 5.** Linear mixed regression models explaining the association between the study

593 variables

Explained effect		Estimate	Standard error	95% Confidence interval (CI)	p-value
Effect of the matrix structure (unstructured vs. structured) on MDI (%)		19.38	5.818	[8.148, 30.612]	0.013
Effect of the MDI (%) on lipolysis extent (%)		8.144	0.856	[6.128, 9.606]	<0.001
Effect of the intestinal conditions on lipolysis extent (%)	Intestinal pH (7 vs. 6)	111.19	29.47	[53.5, 168.9]	<0.001
	Bile salts concentration (10 vs. 1 mM)	147.9	29.48	[90.2, 205.6]	<0.001

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FIGURE LEGENDS

596

597 Figure 1. Matrix degradation index (MDI, %) of the meat products after in vitro

598 digestion with five pancreatic enzyme supplements concentrations (enzyme dose, LU/g

599 fat), classified according to the food matrix structure.

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601 Figure 2. Lipolysis extent (total FFA, mg/g fat) of the meat products after in vitro

602 digestion with five pancreatic enzyme supplements concentrations (enzyme dose, LU/g

603 fat), classified according to the food matrix structure.

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605 Figure 3. Linear mixed regression model plot. (A) Effect of the food matrix (natural or

606 processed) on matrix degradation index (MDI): processed matrices have significantly

607 higher MDI (p = 0.013, 95% CI [8.148, 30.612]). (B) Effect of the MDI on the total

608 FFA released: the higher the MDI, the higher the total FFA released (p <0.001, 95% CI

609 [6.128, 9.606]).

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611 Figure 4. Linear mixed regression model plot. (A) Effect of the intestinal pH (6 or 7) on

612 total FFA: pH 7 allows for significantly higher amount of FFA released (p <0.001, 95%

613 CI [53.466, 168.923]). (B) Effect of the bile salts concentration on the total FFA

614 released: 10mM concentration allows for significantly higher amount of FFA released

615 (p <0.001, 95% CI [90.188, 205.645]).