

1 **Effect of fiber and protein-enriched pasta formulations on satiety-related sensations and**  
2 **afternoon snacking in Italian healthy female subjects**

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27 **ABSTRACT**

28 The objective of the study was to investigate the effect of consuming different fiber and protein-  
29 enriched pasta formulations on satiety response and on mid-afternoon energy intake.

30 Twenty Italian young healthy female subjects participated to a randomized repeated measure study  
31 design developed to evaluate the effect on satiety and energy intake of five different pasta  
32 formulations, i.e. high fiber, high fiber and high protein, high protein from soy, high protein from egg  
33 white, and standard commercial pasta consumed at lunch. The formulations together with a portion  
34 of fruit were consumed on five different occasions followed by an *ad libitum* snack meal proposed  
35 two hours later. Before, immediately after the lunch consumption, and every 30 min until snack time,  
36 satiety sensations were assessed by visual analogue scales. In addition, mid-afternoon energy and  
37 macronutrient intake consumed with the snacks were calculated.

38 Compared to the control pasta, all the formulations significantly affected satiety-related sensations.  
39 Palatability-related attributes of pasta were positively correlated to snack energy intake, whereas  
40 fullness sensation was negatively correlated.

41 Among the formulations tested only the fiber and protein-enriched pasta significantly reduced energy  
42 intake following the *ad libitum* snack consumption ( $p < 0.05$ ).

43 Overall, these findings suggest that pasta enriched with a combination of fiber and protein, might be  
44 effective in the modulation of appetite sensations, thus suggesting a new concept-pasta formulation  
45 for the modulation of eating behavior. These results are interesting considering that pasta is a staple  
46 food in different target groups of the population.

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48 **Keywords:** pasta, fiber, protein, satiety response, energy intake

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## 53 1 INTRODUCTION

54 Food characteristics, including energy density and macronutrient composition, can influence eating  
55 behavior, satiety and consumer liking. In this regard, the role of macronutrients in the modulation of  
56 satiety sensations have been widely studied both in acute and in long term studies (1). As early as the  
57 1980s, Blundell and Burley hypothesized that fiber consumption could limit energy intake by  
58 inducing satiation (short term sensation causing the end of eating) and satiety (involved in the  
59 maintenance of the sensation and determining the subsequent eating event) (2).

60 In this context, the physicochemical properties of fiber (e.g. solubility, viscosity and fermentability)  
61 as well as the characteristics of the food matrix could be considered crucial variables in the regulation  
62 of satiety sensations and energy intake. Viscous fibers have shown to affect subjective appetite and  
63 acute energy intake (3, 4), although the results deriving from different investigations are often  
64 inconsistent(3). In this regard, multiple mechanisms, both in the short and the long term, have been  
65 proposed. Firstly, fiber-rich foods can contribute to a reduced energy density of the whole diet. In  
66 addition, fiber-rich foods are generally less appealing than more energy-dense foods, further affecting  
67 energy intake. Fiber-rich foods generally take longer to chew, so further promoting satiety. However,  
68 the main effect on satiety attributed to fiber is linked to its capacity to increase the fecal bulk by  
69 adsorbing water from the surrounding, increasing viscosity, slowing intestinal transit and,  
70 consequently, delaying gastric emptying (5). These events blunt the absorption of fat and  
71 carbohydrates, extending the period by which these nutrients may exert their effect on satiety through  
72 pre-absorptive mechanisms (5, 6). Moreover, fiber may decrease energy absorption by lowering the  
73 bioavailability of fatty acids and proteins and can be finally fermented in the colon. Derived short  
74 chain fatty acids seem to be involved in the modulation of satiety sensations in the long term (7).

75 As regards proteins, several studies reported their greater satiating potential compared to other  
76 macronutrients. Evidence suggest that the satiating effect of proteins is greater when compared to iso-  
77 energetic amounts of carbohydrates and fat (8, 9). Several mechanisms have been proposed for  
78 justifying such effects. Firstly, protein intake seems to be related to the release of satiety-involved

79 hormones, like peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) (10). On the contrary, no  
80 clear conclusions can be drawn for ghrelin and cholecystokinin due to little or conflicting information  
81 on their contribution to protein-induced satiety (11). Moreover, a high protein diet induces a greater  
82 thermic response compared with a high fat diet, so the role of protein in satiety response could be due  
83 to the effect on energy expenditure that mainly appears in the condition of a high protein diet (11,  
84 12). Finally, the increased concentration of circulating amino acids in the post-absorptive phase, in  
85 addition to a decreased gastric emptying rate, might play an important role in the perception of hunger  
86 and satiety (11, 13, 14). These mechanisms have led to consider that controlled energy intake, in  
87 association with a moderately elevated protein intake, may represent an effective and practical  
88 weight-loss strategy (15, 16). However, in spite of these potential satiating effects of proteins,  
89 conflicting results have been reported in literature depending on the type of food and protein  
90 considered (17). **Vegetal proteins compared to animal proteins, seem to affect satiety. For example,**  
91 **soy proteins have been reported to induce a higher effect on satiety sensations and short-term food**  
92 **intake compared to gelatin, milk, pea or wheat proteins (18). A stronger suppression of hunger and**  
93 **lower food intake was documented following whey proteins compared to milk proteins consumption,**  
94 **while others have found similar results on satiety and food intake(19, 20).**  
95 **The ingestion of animal protein, compared to vegetal protein, have been shown to induce a higher**  
96 **energy expenditure. However, these effects seem to be masked when a mix meal is consumed.**  
97 **In this scenario,** there is a growing interest in investigating the satiating effect of widespread foods  
98 also to better identify the most effective factors involved in satiation and satiety.  
99 Among the most popular and consumed foods there is dried semolina pasta. In fact, it is an easy-to-  
100 use food and its formulation, by means of adequate technological processes, may be easily diversified  
101 to obtain assorted products, characterized by different types and percentages of fiber and protein with  
102 specific potential effect on satiety related sensations. Based on these considerations, the aim of the  
103 present study was to evaluate the effect of consumption of five different pasta formulations on satiety  
104 response and energy intake in healthy young volunteers. The formulations were characterized by

105 different protein quantity and sources, as well as by different fiber content, with the purpose of  
106 increasing understanding on the potential role of ingredients and their combinations to use for the  
107 development of new improved satiating products.

## 108 **2. METHODS**

### 109 **2.1. Participants and Recruitment**

110 Twenty female subjects were selected by advice on bulletin board of the University of Milan. Subjects  
111 were recruited among those who fulfilled the following inclusion criteria: normal weight (BMI<25  
112 kg/m<sup>2</sup>), nonsmokers, no intake of drugs or medications, vitamins or food supplements in the past three  
113 months, similar lifestyle for eating pattern ( $\geq 5$  servings per week of fruit and vegetables,  $\geq 3$  servings  
114 per week of rice or pasta, <3 servings per week of whole grain or fiber-rich foods) and for physical  
115 activity. In addition, only female subjects with a reported liking for the foods included in the study  
116 higher than 5 (“acceptable”) on a scale 1-9 were considered eligible. Conversely, female subjects  
117 were excluded if they: followed specific diets (e.g. vegetarian/vegan or macrobiotic), were pregnant,  
118 lactating or had irregular menstrual cycle, history of diabetes, cardiovascular, hepatic, renal, or  
119 gastrointestinal diseases. The selection of the volunteers was conducted by means of a semi-  
120 quantitative questionnaire focused on eating habits and food preferences (17). A sample size of 18  
121 subjects was calculated, based on previous studies, being sufficient to detect 20% difference (power  
122  $1-\beta=0.80$ ;  $\alpha=0.05$ ) in satiety sensations following pasta intake selected as primary endpoint. For the  
123 study, 20 subjects were selected to reveal a 25% variation in energy intake with the same statistical  
124 power.

125 Selected volunteers signed an approved Informed Consent before participation in the study related  
126 procedures. The research was conducted in accordance with the ethical standards and with the  
127 Helsinki Declaration of 1975 as revised in 2013(21).

### 128 **2.2. Study design**

129 The effect of the different pasta formulations on satiety-related sensations was evaluated by a  
130 randomized repeated measure design. In order to limit the impact of menstrual cycle on eating behavior, 2

131 tests per month were scheduled for each volunteer considering that tests had to be performed 1 week apart.

132 Pasta formulations were randomly served to volunteers on different five occasions, with at least one

133 week among test days. Moreover, an *ad libitum* snack was proposed two hours later to investigate the

134 effect of pasta consumption on mid-afternoon snacking. The experimental design is reported in

135 **Figure 1.**

136 Volunteers were instructed to: i) be fasted since 10 p.m. the evening before the test; ii) consume the

137 same low-fiber breakfast (e.g. milk, coffee and biscuits or croissant) at the same hour (before 8.30

138 a.m.) the morning of the test; iii) do not consume any snack during the morning of the test; iv) do not

139 drink beverages other than water during the whole test. Women were asked to record their breakfast

140 in a food diary to verify compliance with protocol instruction.

141 Once arrived at the laboratory kitchen at 1 p.m., volunteers were seated in a comfortable room until

142 the conclusion of the experiment. To avoid altered satiety-related sensations due to aversive

143 conditions, female subjects were asked to fill a short questionnaire assessing general well-being and

144 hunger feeling at fasting condition. In particular, volunteers were asked whether they felt nauseated,

145 head-ached, sleepy, weakened, starved or full. Scores were rated on 100mm Visual-Analogue Scale

146 (VAS), anchored at either ends with opposite statements (“not at all” and “very much”). Declared

147 sensation of headache, nausea and fullness resulted in a re-scheduling of the test day.

148 Once ascertained the conformity, subjects consumed the whole pasta portion in 15 minutes max, the

149 fruit (150 g apple), and 500 mL plain water was available. VAS questionnaire assessing satiety

150 sensation was completed before (baseline), immediately after the meal consumption and every 30

151 min until snack time. In addition, participants were asked to record their liking for the pasta

152 formulations using VAS ratings, in order to identify any potential confounding effects related with

153 meal palatability.

154 Two hours after lunch consumption, volunteers consumed an *ad libitum* snack test. Sensations related

155 to desire to eat, fullness and satiety were registered before and immediately after snack consumption,

156 and at fixed time points until dinner. Energy and macronutrient intake of consumed snacks were

157 calculated by using both nutritional food labeling and the Food Composition Database for  
158 Epidemiological Studies in Italy (<http://www.bda-ieo.it/>).

159 To examine the sensation ratings over time, the areas under the curves (AUC) calculated with the  
160 trapezoidal rule were used. The analysis was performed with normalized data (compared to basal  
161 values) and data were expressed as cm x min. The satiating efficiency of the pasta was assessed by  
162 the satiety quotient (SQ). SQ, expressed as cm/kcal, is an index obtained by the ratio between  
163 sensation scores and energy intake developed to assess the satiating effect of an eating episode  
164 standardized for unit of intake (weight or energy) (22). The formulas used to evaluate SQ during  
165 lunch (SQ 1), interval (SQ 2) and snack (SQ 3), for each of the three considered sensations (desire to  
166 eat, fullness and satiety), were as follows:

$$\text{SQ 1 (cm/kcal)} = \frac{(\text{Sensation before lunch} - \text{Sensation after lunch})}{\text{Energy content of lunch}} * 100$$

$$\text{SQ 2 (cm/kcal)} = \frac{(\text{Sensation before lunch} - \text{Sensation before snack})}{\text{Energy content of lunch}} * 100$$

$$\text{SQ 3 (cm/kcal)} = \frac{(\text{Sensation before lunch} - \text{Sensation after snack})}{\text{Energy content of lunch} + \text{Energy content of snack}} * 100$$

### 178 2.3. Composition of the lunch

179 Five different dried semolina spaghetti formulations were tested: 1) high fiber (mix of soluble and  
180 insoluble fibers, including resistant starch, oat fiber, inulin); 2) high fiber (same mix) and high protein  
181 (soy + egg); 3) high protein (soy isolate); 4) high protein (egg white) and 5) a standard commercial  
182 pasta as “control”. The nutritional composition of the five pasta formulations is reported in **Table 1**.

183 Lunch was composed of 90 g pasta (raw) served with 100 g of tomato sauce (Barilla G. e R. Fratelli  
184 S.p.A, Italy), and one apple (150 g) as fruit. Apples were peeled, sliced and portioned earlier in the  
185 morning and maintained under vacuum at 4°C until lunch. This meal was developed considering that  
186 consumption of pasta with tomato sauce and one fruit for lunch is a common eating behavior in the  
187 Italian population.

188 The *ad libitum* test snack consisted of an assortment of different foods (sweet, salted and yogurt),  
189 generally selected by the target of subjects involved in the study: 38 g minicake (Barilla G. e R.  
190 Fratelli S.p.A, Italy), 50 g dry snack biscuits (Pavesi-Barilla G. e R. Fratelli S.p.A, Italy), 250 g low-  
191 fat red fruit yogurt (Yomo, Italy) and 60 g low-fat crackers (Barilla G. e R. Fratelli S.p.A, Italy).

192 **Subjects could eat each product as much as they liked.**

193 The nutritional composition of the whole lunch and the *ad libitum* test snack proposed to volunteers  
194 is reported in **Tables 2** and **3**.

#### 195 **2.4. Data Analysis**

196 Statistical analysis was performed by STATISTICA software (Statsoft Inc., Tulsa, OK, US). Changes  
197 in satiety ratings registered for the different pasta formulations were analyzed by means of two-way  
198 ANOVA with type of pasta and time as dependent factors. Energy intake following the *ad libitum*  
199 snack was analyzed by one-way ANOVA assuming energy intake as dependent variable. Data related  
200 to AUCs and SQs were analyzed by one-way ANOVA considering the type of pasta as dependent  
201 variable. For all the experiments, LSD post-hoc analysis was performed setting the significance at  $p$   
202  $<0.05$ .

203 **The relationship among palatability-, satiety-related attributes and snack energy intake was studied**  
204 **by means of the Partial Least Square (PLS) regression (23). Palatability- and satiety-related attributes**  
205 **scores averaged across participants were used as X matrix, whereas snack energy scores averaged**  
206 **across participants were set as Y matrix. Data were standardized (i.e., scaled to unit variance) prior**  
207 **to modeling and full cross validation was chosen as validation method. Correlation loadings plot was**



208 used to find significant variables (>50% explained variance) (24). PLS modeling was performed using  
209 the software The Unscrambler X (CAMO, Norway).

### 210 3. RESULTS

211 Results refer to 19 out of 20 women since one subject voluntarily dropped out before the end of the  
212 study. All the female subjects enrolled were young (mean age:  $28.6 \pm 5.5$  y) and normal weight (BMI:  
213  $20.9 \pm 2.2$  kg/m<sup>2</sup>). All subjects consumed the whole meal provided (i.e. pasta with tomato sauce,  
214 apple) and water intake ( $330 \pm 20$  mL as a mean) was comparable in each test day.

#### 215 3.1. Food palatability

216 **Tables 4** summarizes the subject's ratings related to liking and sensory properties of the 5 pasta  
217 formulations, expressed as mean  $\pm$  SD. In the whole, "high fiber and high protein" and "high protein  
218 (egg white)" pasta resulted less appreciated than other formulations, receiving significantly lower  
219 scores at the questions "How pleasant is this pasta?" and "How difficult was the pasta to eat?" ( $p$   
220  $<0.05$ ). The lower palatability of these formulations was due to several sensory properties,  
221 considering that texture, color and taste scores were lower than those for "high fiber", "high protein  
222 (soy isolate)" and "control" pasta.

#### 223 3.2. Appetite sensations after pasta consumption

224 **Figures 2, 3 and 4** report the rating curves of fullness, desire to eat and satiety sensations over time  
225 and the related AUC values. Ratings were calculated by subtracting baseline ratings.

226 Fullness was significantly higher in all the formulations compared to control pasta ( $p<0.05$ ), except  
227 for the "high fiber" pasta, and remained high up to two hours after pasta consumption.

228 Overall, satiety and desire to eat were not significantly different among the five pasta formulations,  
229 even if LSD test evidenced higher satiety levels ( $p<0.05$ ) following "high fiber" and "high protein  
230 (soy isolate)" pasta compared to control, both immediately after and two hours after pasta  
231 consumption.

232 On the contrary, desire to eat following “high fiber and high protein” and “high protein (soy isolate)”  
233 was significantly lower ( $p < 0.05$ ) both immediately after and two hours after pasta consumption  
234 compared to control pasta formulations.

235 The results obtained on individual ratings were confirmed also by the AUC analysis as shown in  
236 **Figures 1, 2 and 3**. In fact, it was confirmed the effect of the different pasta formulations on fullness  
237 but not on satiety and desire to eat, with “high fiber and high protein”, “high protein (soy isolate)”  
238 and “high protein (egg white)” as the most effective formulations when compared to control pasta.

239 The analysis of satiety quotients was performed to contribute in the understanding of the effect of the  
240 pasta formulations in the control of satiety sensations.

241 The quotients related to fullness resulted significantly higher for all formulations compared to control  
242 pasta both immediately after lunch and over the subsequent two hours as indicated in **Table 5**. “High  
243 fiber and high protein” pasta, but not the other pasta formulations, induced significantly lower desire  
244 to eat **than** control pasta both after lunch and after snack consumption, while only “high fiber” pasta  
245 showed a higher satiety quotients compared to control.

### 246 **3.3. Energy intake**

247 **Table 6** shows the energy **and macronutrient** intake registered after the “*ad libitum*” snack  
248 consumption two hours after lunch, **as well as the amount of each food consumed**. Statistical analysis  
249 shows that only “high fiber and high protein” formulation significantly influenced **the energy intake**  
250 **at snack-time** if compared to the other formulations.

### 251 **3.4. Relationship among palatability-, satiety-related attributes and snack energy intake**

252 **The Scores and Correlation loading plots from PLS modeling are reported in Figure 5 A and B,**  
253 **respectively. The first factor explains respectively the 58% and 56% of the variation in X and Y,**  
254 **while the second factor accounts for respectively the 7% and 39%.**

255 **In the Scores plot (Figure 5A), pasta formulations near to each other are considered as similar, while**  
256 **pasta formulations positioned far from each other are different for the selected variables. Similarly,**  
257 **in the Correlation loading plot, variables laying near the Y variable (Energy, indicated in red in Figure**

258 5B) give a positive contribution to the Y estimate, while variables located in the opposite part of the  
259 graph contribute negatively to the Y estimate.

260 The Correlation loadings plot shows that Palatability-related attributes (except difficulty to eat) are  
261 positively correlated to snack energy intake, whereas fullness variation and AUC as well as difficulty  
262 to eat are negatively correlated to snack energy intake. The Correlation loadings plot also shows that  
263 satiety and desire to eat (both variation and AUC) play a marginal role in predicting snack energy  
264 intake.

265 The comparison of the Scores (Figure 5A) and Correlation loadings Plots (Figure 5B) shows that the  
266 “high fiber”, “high protein (soy isolate)” and “control pasta” formulations contribute positively to  
267 energy intake and are more liked by participants. Conversely, “high fiber and high protein” and “high  
268 protein (egg white)” formulations contribute negatively to snack energy intake, are less liked, are  
269 more difficult to eat and show high ratings of Fullness variation and AUC.

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#### 271 **4. DISCUSSION**

272 The modulation of appetite through an increased satiation is getting increased attention, and its crucial  
273 role in weight management is supported by the consideration that, as stated by the European Food  
274 Safety Authority, health claims on changes in appetite ratings can be made in the context of reducing  
275 body weight (25, 26).

276 In the current study, we aimed to evaluate the effect of innovative pasta formulations on satiety  
277 response and on mid-afternoon energy intake in an Italian group of young healthy **women**.

278 The first consideration rising from the results is a reduced **palatability** of some pasta formulations as  
279 rated by these regular consumers. In fact, “high protein (egg white)” and “high fiber (mix of soluble  
280 and insoluble fibers) and high protein (soy+egg)” pasta resulted less appreciated than the other  
281 formulations, indicating that egg protein are likely to negatively affect the sensory properties of pasta,  
282 except for the appearance.

283 All the pasta formulations compared to the control pasta significantly affected satiety-related  
284 sensations; however, the differences in palatability among products seem to be an important  
285 modulatory factor.

286 Considering the different sensations, fullness was significantly higher in all the formulations than  
287 control pasta except for the “high fiber” pasta. As previously reported, fullness is the easiest sensation  
288 to identify because strictly related to physiological feelings (27, 28). Conversely, desire to eat and  
289 satiety did not differ after pasta consumption, plausibly due to the simultaneous implication of both  
290 physiological and psychological aspects.

291 “High fiber and high protein” pasta resulted the only formulation able to reduce energy intake during  
292 the *ad libitum* snacking. It has been reported that the effect of protein on satiety could be attributed  
293 to the increased secretion of gastrointestinal hormones regulating appetite, including PYY,  
294 cholecystokinin, glucagon and GLP-1, together with a reduction in circulating ghrelin levels (13, 14,  
295 29) or the effect on thermogenesis (30). However, such mechanisms can explain better long term  
296 effect of protein not considered in our study (i.e. sensations were followed for 2 h from pasta intake).  
297 Conversely, we found that “high fiber and high protein” formulation contributed negatively to snack  
298 energy intake, probably because less liked, more difficult to eat and able to increase fullness.

299 As regard the effect of food containing both protein and fiber on satiety, it has not been fully  
300 understood. Lee and coworkers reported a significantly higher self-reported satiety in subjects  
301 consuming lupin kernel flour– enriched bread at breakfast (31). However, the same group failed to  
302 demonstrate a significant effect on body weight in overweight men and women following a long-term  
303 *ad libitum* diet added with this enriched bread (32).

304 Some authors also reported that animal or vegetal proteins might exert different specific effects in the  
305 modulation of satiety sensations and energy intake (33, 34). It may be related to a diverse stimulation  
306 of amino acid synthesis and oxidation (35) probably due to the different digestion rate of these two  
307 protein classes. However, in the present short term study, we could not demonstrate differences on  
308 satiety-sensations between the two pasta formulations made with animal or vegetal protein.

309 As regards fiber, it has been shown that it can affect food consumption through an effect on gastric  
310 relaxation. Moreover, **in the long term** fermentable fiber can influence post-prandial satiety by the  
311 formation of propionic and acetic acid that can signal the secretion of gastrointestinal hormones like  
312 GLP-1(36).

313 In our experimental conditions (**i.e. evaluation in the short-term**), the lack of effect of the “high fiber”  
314 pasta on fullness might be at least partially ascribed to the lower energy intake of this formulation.  
315 Indeed, when **women** were asked to consume a larger portion of this pasta (i.e. 130 g vs. 90 g) to get  
316 the same energy intake of the other formulations the rating of fullness was significantly higher than  
317 that of control pasta (data not shown).

318 These results are in agreement with a previous study finding that consumption of fiber-rich  
319 wholemeal breads increased satiety ratings compared to refined breads without significant effect on  
320 subsequent energy intake (37). Conversely, Berti et al. (38) found higher satiating efficiency indices  
321 for pasta and breads made with alternative fiber-rich crop foods (i.e. oat and buckwheat) compared  
322 to the wheat counterparts.

323 Interestingly, Korczak et al. (39) did not find a higher satiety response after consumption of “high  
324 protein” or “high fiber” pasta consumed as ready-to-eat meals, compared to traditional pasta. The  
325 conflicting results could be due to the different types and doses of fiber, as well as to subjects and  
326 study design used with respect to our investigation (**i.e. where only women with BMI in the normal  
327 range have been considered**). In addition, differences in product preparation (i.e. the cooking  
328 procedure) can greatly affect the results on acceptability and eating behavior.

329 In the present study, we also considered the satiety quotients, introduced by Green et al. (22) to  
330 standardize the satiety sensations in relation to the energy intake. Satiety quotients can give a measure  
331 of the extent to which the food eaten during the eating episode reduced subjective appetite per unit of  
332 intake. Our results on satiety quotients did not differ from those on satiety-sensation ratings and AUC.

333 As an exception, quotient for satiety and fullness following “high fiber” pasta were significantly lower  
334 probably due to the lower energy content compared to the other formulations.

335 As regards mid-afternoon snacking, we found that only the “high fiber and high protein” pasta  
336 formulation had a significant effect on subsequent energy intake. These results are partially in contrast  
337 with those reported by the above-mentioned study by Korczak et al. (39), who did not find differences  
338 among the pasta treatments for snacking. In that study, as discussed by the authors, since the majority  
339 of subjects were students with limited budget, they ended up consuming most of the snacks regardless  
340 of any satiety-related sensations. In addition, differently from our study, the blend of snacks included  
341 highly palatable and appealing foods, thus probably contributing to the failure in the control of food  
342 intake. Finally, differences among findings can be ascribed to the different composition of pasta  
343 formulations as well as to the fact that our study was performed in a group of Italian volunteers for  
344 whom pasta is consumed on a daily basis.

345 Our work has several strengths. Firstly, the use of a cross-over design allowed the subjects to act as  
346 their own control, so reducing the impact of inter-individual variability. Moreover, the selection of  
347 snack was performed considering the characteristics of volunteers and comprised a blend of foods  
348 that are typically consumed as mid-afternoon snack. Regarding limitations, we used a short term  
349 protocol to evaluate the impact of pasta formulations on eating behavior thus we cannot exclude  
350 different effects in the long term. Moreover, it is important to consider that all the subjects enrolled  
351 in our study were young and normal weight women, in which cognitive factors could play an  
352 important role on eating behavior and we did not specifically ascertain the presence of restraint eating.  
353 Therefore, our results may be not overall translated to the general population.

## 354 CONCLUSIONS

355 In the present study, we investigated the effect of consumption of five different pasta formulations  
356 made with protein from different sources, alone or in combination with fiber, on satiety response and  
357 energy intake in young healthy women. Our results and those from the literature reveal that many  
358 factors must be taken into consideration to better detail the effect of a food on satiety and eating  
359 behavior including cultural and attitudinal characteristics. In particular, from our results new pasta  
360 formulations with combinations of fiber and protein seem to be effective in the modulation of appetite

361 sensations and subsequent energy intake, at least in our condition of preparation and specific target  
362 population (i.e. young women). In this regard, it is important to consider that sensory properties of  
363 foods seem to be critical determinants of eating behavior, thus a fundamental variable for the design  
364 of products tailored to different types of consumers.

365

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485 Table 1. Nutritional composition of different pasta formulations, tomato sauce and apple used in the study. Data are expressed per 100g and per % contribution total  
 486 energy.

	ENERGY	PROTEIN	FAT	CARBOHYDRATE	FIBER
	kcal	g / % kcal	g / % kcal	g / % kcal	g / % kcal
<b>PASTA FORMULATION</b>					
High fiber	328	13.0 / 15.9	2.5 / 6.9	53.8 / 65.6	19.2 / 11.7
High fiber and high protein	335	20.5 / 24.5	2.5 / 6.7	50.2 / 59.9	15.0 / 9.0
High protein (soy isolate)	360	22.0 / 24.4	2.5 / 6.3	60.4 / 67.1	3.5 / 1.9
High protein (egg white)	357	21.0 / 23.5	2.0 / 5.0	62.4 / 69.9	2.5 / 1.4
Control	363	14.5 / 16.0	2.5 / 6.2	69.2 / 76.3	2.5 / 1.4
TOMATO SAUCE	73	1.2 / 6.6	3.5 / 43.2	7.8 / 42.7	2.5 / 6.8
APPLE	51	0.2 / 1.6	0.3 / 5.3	11.0 / 86.3	2.0 / 7.8

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492 Table 2. Nutritional composition of lunch (90g pasta with 100g tomato sauce and 150g apple) proposed to volunteers. Data are expressed per kcal and grams and per  
 493 % contribution total energy.

LUNCH	ENERGY	PROTEIN	FAT	CARBOHYDRATE	FIBER
	kcal	g / % kcal	g / % kcal	g / % kcal	g / % kcal
High fiber	445	13.2/11.7	6.2/ 12.5	72.7/ 65.3	22.8 / 10.2
High fiber and high protein	452	20.0 / 17.7	6.2 / 12.3	69.5 / 61.5	19.0 / 8.4
High protein (soy isolate)	473	21.3 / 18.0	6.2 / 11.8	78.7 / 66.6	8.7 / 3.7
High protein (egg white)	471	20.4 / 17.3	5.8 / 11.1	80.5 / 68.4	7.8 / 3.3
Control	476	14.6 / 12.3	6.2 / 11.7	86.6 / 72.8	7.8 / 3.3

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502 Table 3. Nutrient composition of the ad libitum snack proposed to volunteers. Data are expressed per 100g.

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SNACK	ENERGY (kcal)	PROTEIN (g)	FAT (g)	SATURATED (g)	CARBOHYDRATE (g)	SUGARS (g)	FIBER (g)
Minicake	324	7.3	9.3	3.1	52.5	18.0	3.0
Low-fat crackers	408	11.0	7.0	1.2	72.3	2.5	6.0
Dry snack biscuits	395	7.0	3.5	1.2	82.6	50.0	2.3
Low fat red fruit yogurt	75	3.8	0.1	0.07	13.9	13.9	0.1

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509 Table 4. Ratings (cm) of pasta perceived characteristics registered by volunteers (n=19). Values are expressed as mean  $\pm$  SD. <sup>a,b,c</sup>Data with different letters within the  
 510 same column indicate significant difference determined by LSD post-hoc analysis ( $p < 0.05$ ).

PASTA	How pleasant is this pasta (palatability)?	How difficult was the pasta to eat?	Do you want to eat pasta any more (willingness)?	How do you judge the appearance of this pasta?	How do you judge the color of this pasta?	How pleasant is the taste of this pasta?	How do you judge the texture of this pasta?
High fiber	6.2 $\pm$ 2.6 <sup>a</sup>	1.3 $\pm$ 1.9 <sup>a</sup>	2.7 $\pm$ 2.3 <sup>a</sup>	5.5 $\pm$ 2.0 <sup>ab</sup>	5.5 $\pm$ 2.2 <sup>ab</sup>	6.3 $\pm$ 2.2 <sup>a</sup>	6.1 $\pm$ 2.4 <sup>a</sup>
High fiber and high protein	3.6 $\pm$ 2.8 <sup>b</sup>	3.5 $\pm$ 3.7 <sup>b</sup>	1.6 $\pm$ 1.9 <sup>bc</sup>	5.1 $\pm$ 2.3 <sup>a</sup>	4.9 $\pm$ 2.2 <sup>a</sup>	3.9 $\pm$ 2.8 <sup>b</sup>	3.8 $\pm$ 3.2 <sup>b</sup>
High protein (soy isolate)	6.7 $\pm$ 1.9 <sup>a</sup>	1.3 $\pm$ 2.4 <sup>a</sup>	2.2 $\pm$ 2.1 <sup>ac</sup>	5.9 $\pm$ 2.2 <sup>ab</sup>	5.9 $\pm$ 2.4 <sup>ab</sup>	6.5 $\pm$ 1.9 <sup>a</sup>	6.5 $\pm$ 2.4 <sup>a</sup>
High protein (egg white)	2.5 $\pm$ 2.6 <sup>b</sup>	6.3 $\pm$ 3.1 <sup>b</sup>	0.7 $\pm$ 1.3 <sup>b</sup>	5.3 $\pm$ 2.5 <sup>ab</sup>	4.9 $\pm$ 2.8 <sup>a</sup>	3.4 $\pm$ 2.8 <sup>b</sup>	2.4 $\pm$ 3.3 <sup>b</sup>
Control	6.2 $\pm$ 2.4 <sup>a</sup>	1.3 $\pm$ 1.7 <sup>a</sup>	2.6 $\pm$ 2.4 <sup>ac</sup>	6.5 $\pm$ 2.3 <sup>b</sup>	6.6 $\pm$ 1.7 <sup>b</sup>	6.3 $\pm$ 2.4 <sup>a</sup>	6.0 $\pm$ 2.8 <sup>a</sup>

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515 Table 5. Satiety quotients related to fullness, desire to eat and satiety sensations for each pasta. Values are  
 516 expressed as mean  $\pm$  SD. *<sup>a,b</sup>Data with different letters within the same column indicate significant difference*  
 517 *determined by LSD post-hoc analysis (p<0.05).*

SENSATION (cm/kcal)	Lunch	Break	Snack
<b>FULLNESS</b>			
High fiber	1.64 $\pm$ 0.69 <sup>a</sup>	0.86 $\pm$ 0.48 <sup>a</sup>	0.94 $\pm$ 0.51 <sup>ab</sup>
High fiber and high protein	1.69 $\pm$ 0.63 <sup>a</sup>	0.89 $\pm$ 0.51 <sup>a</sup>	1.00 $\pm$ 0.40 <sup>a</sup>
High protein (soy isolate)	1.50 $\pm$ 0.6 <sup>a</sup>	0.83 $\pm$ 0.40 <sup>a</sup>	0.86 $\pm$ 0.30 <sup>ab</sup>
High protein (egg white)	1.48 $\pm$ 0.52 <sup>a</sup>	0.82 $\pm$ 0.47 <sup>a</sup>	0.93 $\pm$ 0.29 <sup>ab</sup>
Control	1.16 $\pm$ 0.58 <sup>b</sup>	0.54 $\pm$ 0.51 <sup>b</sup>	0.81 $\pm$ 0.28 <sup>b</sup>
<b>DESIRE TO EAT</b>			
High fiber	1.40 $\pm$ 0.69 <sup>ab</sup>	1.00 $\pm$ 0.63 <sup>a</sup>	1.05 $\pm$ 0.35 <sup>ab</sup>
High fiber and high protein	1.61 $\pm$ 0.47 <sup>b</sup>	1.13 $\pm$ 0.64 <sup>a</sup>	1.18 $\pm$ 0.35 <sup>b</sup>
High protein (soy isolate)	1.40 $\pm$ 0.54 <sup>ab</sup>	1.02 $\pm$ 0.49 <sup>a</sup>	1.05 $\pm$ 0.35 <sup>ab</sup>
High protein (egg white)	1.29 $\pm$ 0.56 <sup>ab</sup>	0.84 $\pm$ 0.61 <sup>a</sup>	1.00 $\pm$ 0.41 <sup>ab</sup>
Control	1.20 $\pm$ 0.40 <sup>a</sup>	0.82 $\pm$ 0.55 <sup>a</sup>	0.97 $\pm$ 0.30 <sup>a</sup>
<b>SATIETY</b>			
High fiber	1.60 $\pm$ 0.66 <sup>b</sup>	0.90 $\pm$ 0.52 <sup>b</sup>	1.00 $\pm$ 0.39 <sup>a</sup>
High fiber and high protein	1.36 $\pm$ 0.65 <sup>ab</sup>	0.75 $\pm$ 0.56 <sup>ab</sup>	0.99 $\pm$ 0.39 <sup>a</sup>
High protein (soy isolate)	1.43 $\pm$ 0.41 <sup>ab</sup>	0.84 $\pm$ 0.44 <sup>ab</sup>	0.99 $\pm$ 0.33 <sup>a</sup>
High protein (egg white)	1.32 $\pm$ 0.58 <sup>ab</sup>	0.68 $\pm$ 0.62 <sup>ab</sup>	0.96 $\pm$ 0.34 <sup>a</sup>
Control	1.24 $\pm$ 0.42 <sup>a</sup>	0.60 $\pm$ 0.50 <sup>a</sup>	0.93 $\pm$ 0.31 <sup>a</sup>

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524 Table 6. Energy and macronutrient intake through snacks consumed 2 hours after pasta consumption (n=19). Values are expressed as mean  $\pm$  SD. <sup>a,b</sup>Data with  
 525 different letters within the same column indicate significant difference determined by LSD post-hoc analysis (p<0.05).

PASTA	ENERGY (Kcal) AND MACRONUTRIENTS (g)							FOOD CHOICE (g/ml)				
	ENERGY	PROTEIN	FAT	SATURATED	CARBOHYDRATE	SUGARS	FIBER	MINICAKE	LOW-FAT CRACKERS	DRY SNACK BISCUITS	LOW FAT RED FRUIT	WATER YOGURT
HF	231 $\pm$ 67 <sup>b</sup>	7.23 $\pm$ 3.0	2.8 $\pm$ 1.5	0.8 $\pm$ 0.4	42.9 $\pm$ 13.2	24.5 $\pm$ 12.7	1.6 $\pm$ 0.8	13.7 $\pm$ 18.2	12.2 $\pm$ 14.4	15.8 $\pm$ 17.9	99.9 $\pm$ 83.7	228 $\pm$ 67
HFHP	219 $\pm$ 93 <sup>a</sup>	7.5 $\pm$ 3.0	2.4 $\pm$ 2.0	0.7 $\pm$ 0.7	40.4 $\pm$ 17.4	25.2 $\pm$ 12.2	1.4 $\pm$ 0.8	12.9 $\pm$ 22.5	10.3 $\pm$ 11.6	10.8 $\pm$ 14.9	123.8 $\pm$ 80.2	235 $\pm$ 172
HP (soy isolate)	263 $\pm$ 91 <sup>b</sup>	8.4 $\pm$ 3.8	3.5 $\pm$ 1.6	1.1 $\pm$ 0.5	48.0 $\pm$ 12.7	26.5 $\pm$ 13.6	1.9 $\pm$ 0.8 <sup>a</sup>	21.1 $\pm$ 19.4	14.3 $\pm$ 14.7	13.0 $\pm$ 14.7	113.8 $\pm$ 87.5	236 $\pm$ 154
HP (egg white)	234 $\pm$ 96 <sup>b</sup>	7.5 $\pm$ 3.2	2.9 $\pm$ 2.1	0.9 $\pm$ 0.7	43.2 $\pm$ 18.2	25.4 $\pm$ 13.9	1.6 $\pm$ 0.9	16.9 $\pm$ 26.8	10.8 $\pm$ 15.0	13.7 $\pm$ 17.0	109.4 $\pm$ 73.3	232 $\pm$ 132
Control	267 $\pm$ 106 <sup>b</sup>	8.6 $\pm$ 3.9	3.6 $\pm$ 2.1	1.1 $\pm$ 0.7	48.7 $\pm$ 19.1	26.9 $\pm$ 14.0	1.9 $\pm$ 1.1	21.4 $\pm$ 23.1	15.0 $\pm$ 18.2	12.0 $\pm$ 17.2	119.8 $\pm$ 78.6	223 $\pm$ 145

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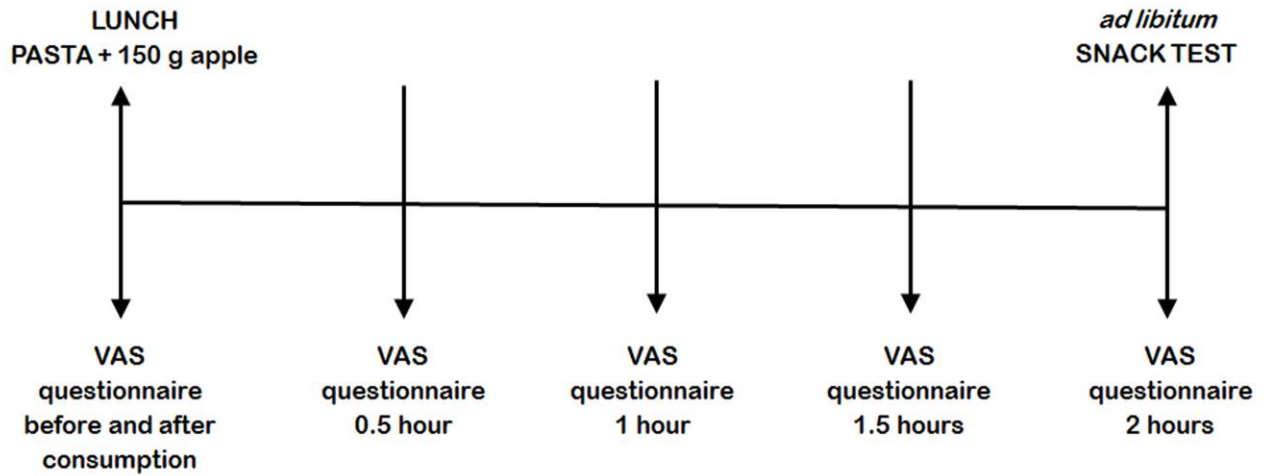
527 Legend: HF="High-fiber" pasta; HFHP="High fiber and high protein" pasta; HPsoy="High protein (soy isolate)" pasta; HPegg="High protein (egg white)" pasta;  
 528 Control="Control" pasta

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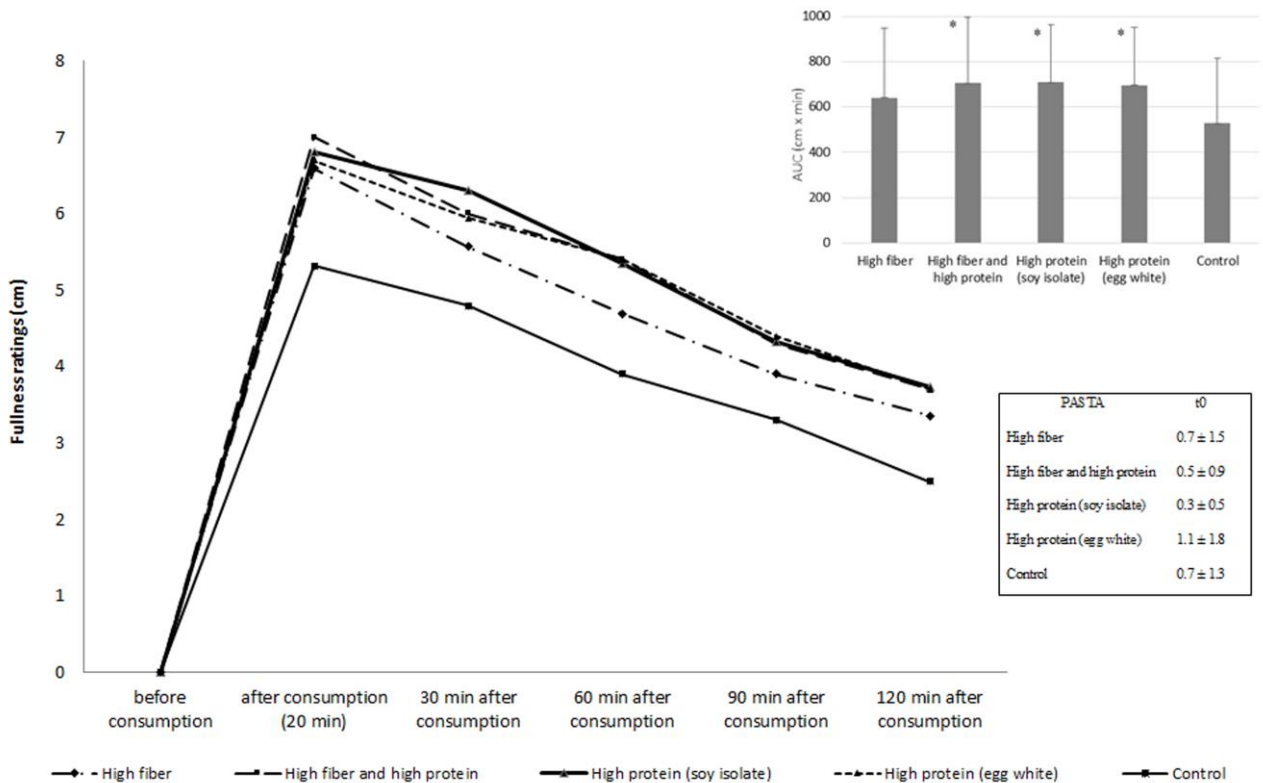
531 **FIGURE CAPTION**

532 Fig.1 Study design



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534 Fig.2 Rating curves of fullness over time and related AUC values (in the box on the top right). Asterisk  
 535 indicates significant differences between each formulations and control pasta ( $p < 0.05$ ). In the box on the  
 536 bottom right, ratings of fullness before the lunch ( $t_0$ ) are reported.

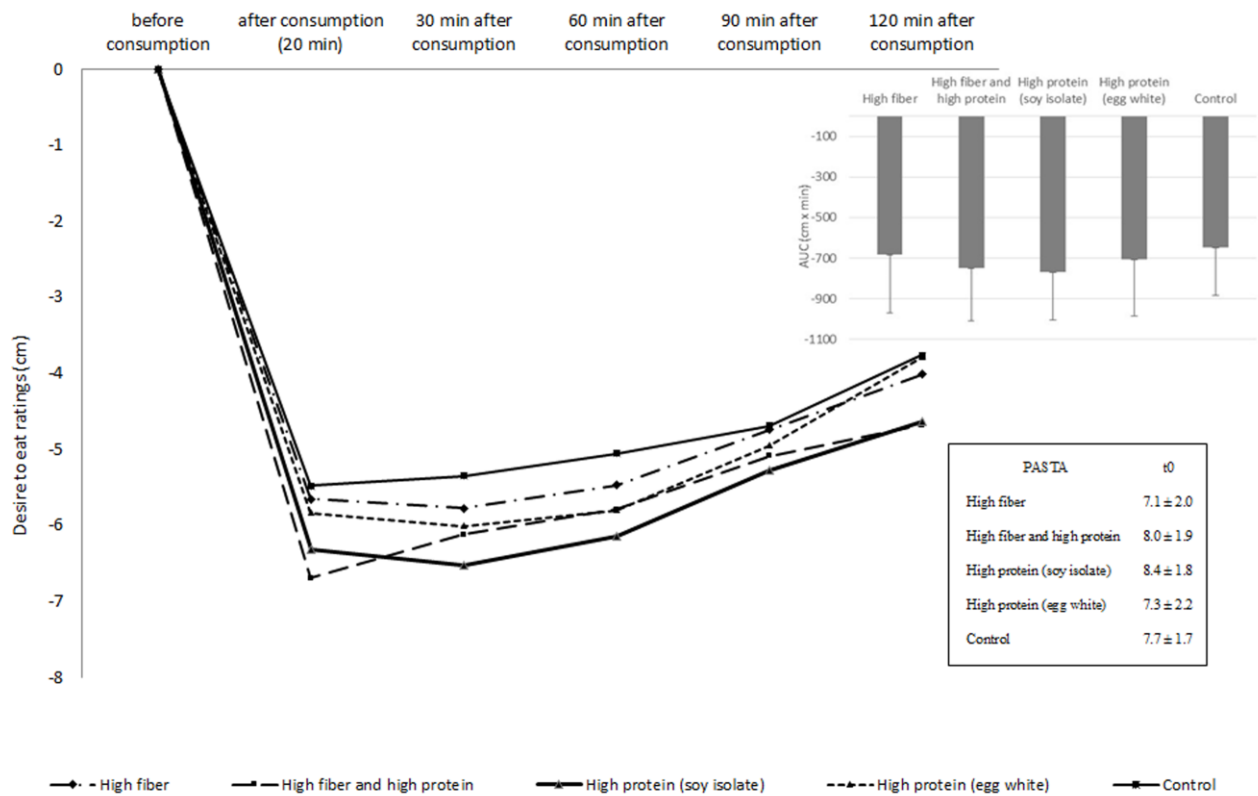


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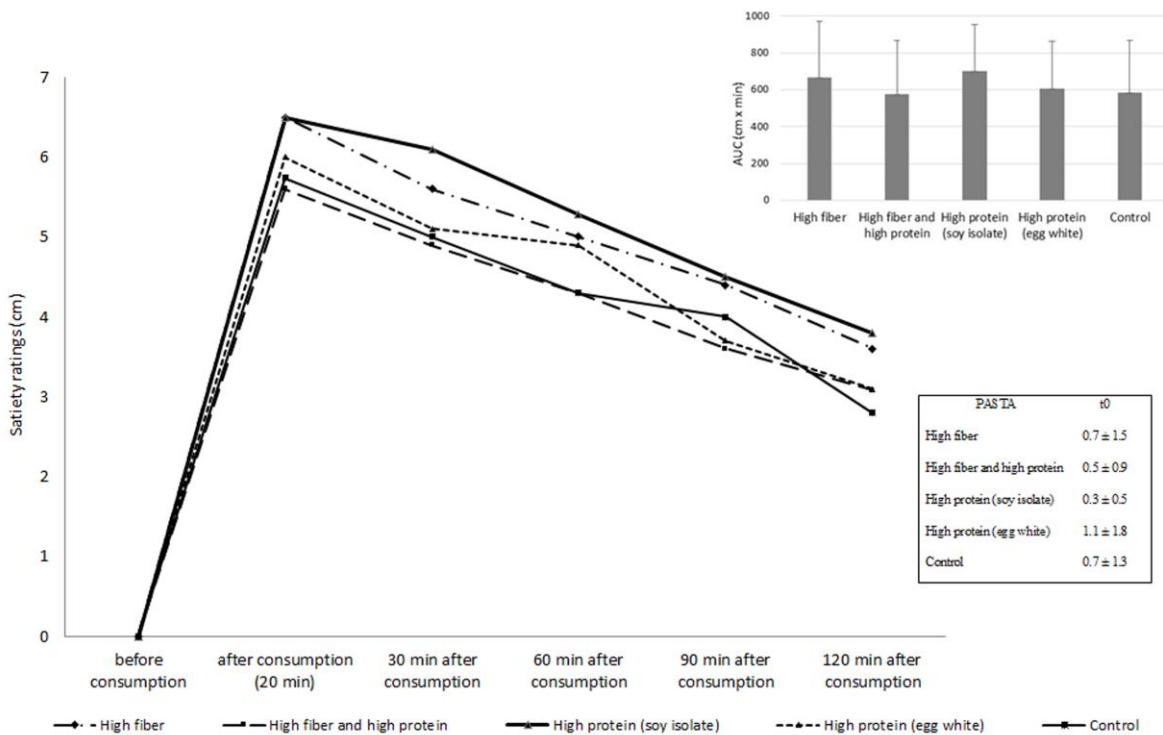
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540 Fig.3 Rating curves of desire to eat over time and related AUC values (in the box on the top right). In the box  
 541 on the bottom right, ratings of desire to eat before the lunch (t0) are reported.



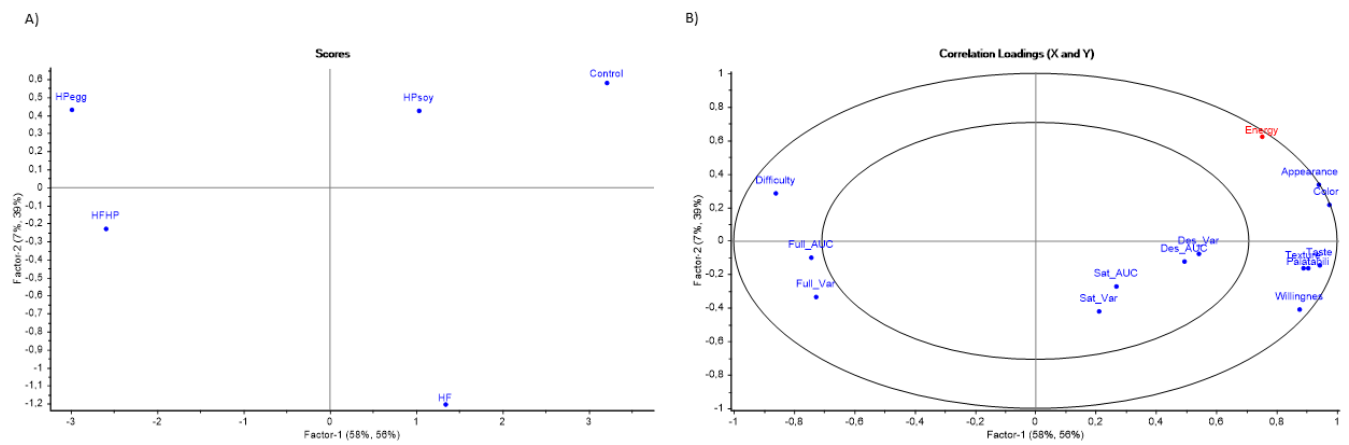
542  
 543 Fig.4 Rating curves of satiety over time and related AUC values (in the box on the top right). In the box on the  
 544 bottom right, ratings of satiety before the lunch (t0) are reported.



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546 Fig.5 Scores Plot (A) and Correlation Loadings Plot (B) obtained by the PLSR model of the five pasta  
 547 formulations based on palatability and related sensory attributes snack energy data.  
 548 Concentric circles in b show the loci of 100 and 50% explained variance. Legend: HF="High-fiber" pasta;  
 549 HPHP="High fiber and high protein" pasta; HPsoy="High protein (soy isolate)" pasta; HPegg="High protein  
 550 (egg white)" pasta; Control="Control pasta"; Full\_AUC=Fullness AUC; Full\_Var=Fullness variation;  
 551 Sat\_AUC=Satiety AUC; Sat\_Var=Satiety variation; Des\_AUC=Desire to eat AUC; Des\_Var=Desire to eat  
 552 variation; Difficulty=Difficulty to eat; Willingness=Willingness to eat; Energy=Snack energy intake.  
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Figure 5



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