



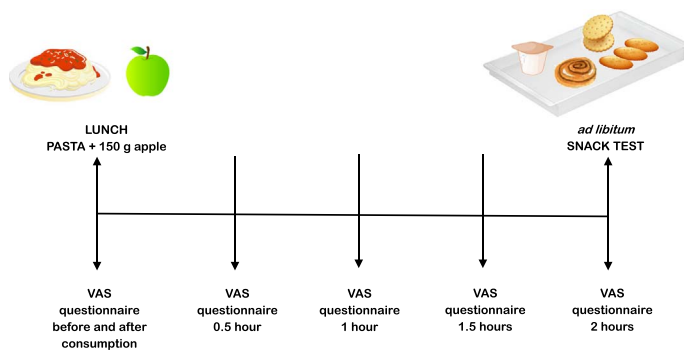
## Effect of fiber and protein-enriched pasta formulations on satiety-related sensations and afternoon snacking in Italian healthy female subjects

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### GRAPHICAL ABSTRACT



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### ABSTRACT

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

Twenty Italian young healthy female subjects participated to a randomized repeated measure study design developed to evaluate the effect on satiety and energy intake of five different pasta formulations, i.e. high fiber, high fiber and high protein, high protein from soy, high protein from egg white, and standard commercial pasta consumed at lunch. The formulations together with a portion of fruit were consumed on five different occasions followed by an ad libitum snack meal proposed 2 h later. Before, immediately after the lunch consumption, and every 30 min until snack time, satiety sensations were assessed by visual analogue scales. In addition, mid-afternoon energy and macronutrient intake consumed with the snacks were calculated.

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

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

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

## 1. Introduction

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

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

As regards proteins, several studies reported their greater satiating potential compared to other macronutrients. Evidence suggest that the satiating effect of proteins is greater when compared to iso-energetic amounts of carbohydrates and fat [8,9]. Several mechanisms have been proposed for justifying such effects. Firstly, protein intake seems to be related to the release of satiety-involved hormones, like peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) [10]. On the contrary, no clear conclusions can be drawn for ghrelin and cholecystokinin due to little or conflicting information on their contribution to protein-induced satiety [11]. Moreover, a high protein diet induces a greater thermic response compared with a high fat diet, so the role of protein in satiety response could be due to the effect on energy expenditure that mainly

appears in the condition of a high protein diet [11,12]. Finally, the increased concentration of circulating amino acids in the post-absorptive phase, in addition to a decreased gastric emptying rate, might play an important role in the perception of hunger and satiety [11,13,14]. These mechanisms have led to consider that controlled energy intake, in association with a moderately elevated protein intake, may represent an effective and practical weight-loss strategy [15,16]. However, in spite of these potential satiating effects of proteins, conflicting results have been reported in literature depending on the type of food and protein considered [17]. Vegetal proteins compared to animal proteins, seem to affect satiety. For example, soy proteins have been reported to induce a higher effect on satiety sensations and short-term food intake compared to gelatin, milk, pea or wheat proteins [18]. A stronger suppression of hunger and lower food intake was documented following whey proteins compared to milk proteins consumption, while others have found similar results on satiety and food intake [19,20].

The ingestion of animal protein, compared to vegetal protein, have been shown to induce a higher energy expenditure. However, these effects seem to be masked when a mix meal is consumed.

In this scenario, there is a growing interest in investigating the satiating effect of widespread foods also to better identify the most effective factors involved in satiation and satiety.

Among the most popular and consumed foods there is dried semolina pasta. In fact, it is an easy-to-use food and its formulation, by means of adequate technological processes, may be easily diversified to obtain assorted products, characterized by different types and percentages of fiber and protein with specific potential effect on satiety related sensations. Based on these considerations, the aim of the present study was to evaluate the effect of consumption of five different pasta formulations on satiety response and energy intake in healthy young volunteers. The formulations were characterized by different protein quantity and sources, as well as by different fiber content, with the purpose of increasing understanding on the potential role of ingredients and their combinations to use for the development of new improved satiating products.

## 2. Methods

### 2.1. Participants and recruitment

Twenty female subjects were selected by advice on bulletin board of the University of Milan. Subjects were recruited among those who fulfilled the following inclusion criteria: normal weight (BMI < 25 kg/m<sup>2</sup>), nonsmokers, no intake of drugs or medications, vitamins or food supplements in the past three months, similar lifestyle for eating pattern

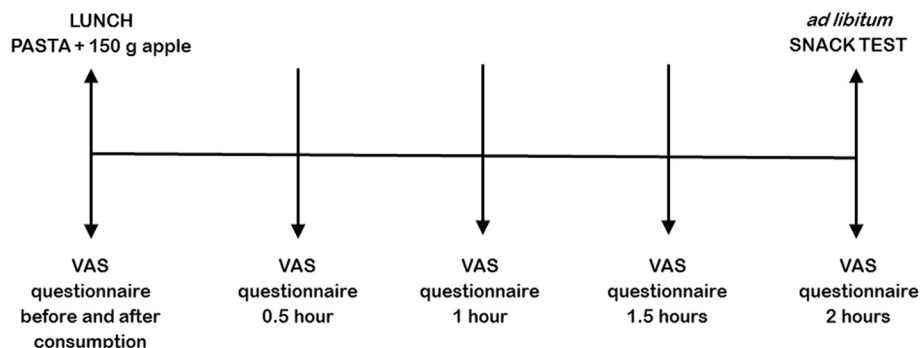


Fig. 1. Study design.

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

	Energy kcal	Protein g/% kcal	Fat g/% kcal	Carbohydrate g/% kcal	Fiber g/% kcal
Pasta formulation					
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

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

Lunch	Energy kcal	Protein g/% kcal	Fat g/% kcal	Carbohydrate g/% kcal	Fiber 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

( $\geq 5$  servings per week of fruit and vegetables,  $\geq 3$  servings per week of rice or pasta,  $< 3$  servings per week of whole grain or fiber-rich foods) and for physical activity. In addition, only female subjects with a reported liking for the foods included in the study higher than 5 (“acceptable”) on a scale 1–9 were considered eligible. Conversely, female subjects were excluded if they: followed specific diets (e.g. vegetarian/vegan or macrobiotic), were pregnant, lactating or had irregular menstrual cycle, history of diabetes, cardiovascular, hepatic, renal, or gastrointestinal diseases. The selection of the volunteers was conducted by means of a semi-quantitative questionnaire focused on eating habits and food preferences [17]. A sample size of 18 subjects was calculated, based on previous studies, being sufficient to detect 20% difference (power  $1 - \beta = 0.80$ ;  $\alpha = 0.05$ ) in satiety sensations following pasta intake selected as primary endpoint. For the study, 20 subjects were selected to reveal a 25% variation in energy intake with the same statistical power.

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

## 2.2. Study design

The effect of the different pasta formulations on satiety-related sensations was evaluated by a randomized repeated measure design. In

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

Snack	Energy (kcal)	Protein (g)	Fat (g)	Saturated (g)	Carbohydrate (g)	Sugars (g)	Fiber (g)
Mimicake	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

order to limit the impact of menstrual cycle on eating behavior, 2 tests per month were scheduled for each volunteer considering that tests had to be performed 1 week apart. Pasta formulations were randomly served to volunteers on different five occasions, with at least one week among test days. Moreover, an ad libitum snack was proposed 2 h later to investigate the effect of pasta consumption on mid-afternoon snacking. The experimental design is reported in Fig. 1.

Volunteers were instructed to: i) be fasted since 10 p.m. the evening before the test; ii) consume the same low-fiber breakfast (e.g. milk, coffee and biscuits or croissant) at the same hour (before 8.30 a.m.) the morning of the test; iii) do not consume any snack during the morning of the test; iv) do not drink beverages other than water during the whole test. Women were asked to record their breakfast in a food diary to verify compliance with protocol instruction.

Once arrived at the laboratory kitchen at 1 p.m., volunteers were seated in a comfortable room until the conclusion of the experiment. To avoid altered satiety-related sensations due to aversive conditions, female subjects were asked to fill a short questionnaire assessing general well-being and hunger feeling at fasting condition. In particular, volunteers were asked whether they felt nauseated, head-ached, sleepy, weakened, starved or full. Scores were rated on 100 mm Visual-Analogue Scale (VAS), anchored at either ends with opposite statements (“not at all” and “very much”). Declared sensation of headache, nausea and fullness resulted in a re-scheduling of the test day.

Once ascertained the conformity, subjects consumed the whole pasta portion in 15 min max, the fruit (150 g apple), and 500 mL plain water was available. VAS questionnaire assessing satiety sensation was completed before (baseline), immediately after the meal consumption and every 30 min until snack time. In addition, participants were asked to record their liking for the pasta formulations using VAS ratings, in order to identify any potential confounding effects related with meal palatability.

Two hours after lunch consumption, volunteers consumed an ad libitum snack test. Sensations related to desire to eat, fullness and satiety were registered before and immediately after snack consumption, and at fixed time points until dinner. Energy and macronutrient intake of consumed snacks were calculated by using both nutritional food labeling and the Food Composition Database for Epidemiological Studies in Italy (<http://www.bda-ieo.it/>).

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

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

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

**Table 4**  
Ratings (cm) of pasta perceived characteristics registered by volunteers (n = 19). Values are expressed as mean ± SD.

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 ± 2.6 <sup>a</sup>	1.3 ± 1.9 <sup>a</sup>	2.7 ± 2.3 <sup>a</sup>	5.5 ± 2.0 <sup>ab</sup>	5.5 ± 2.2 <sup>ab</sup>	6.3 ± 2.2 <sup>a</sup>	6.1 ± 2.4 <sup>a</sup>
High fiber and high protein	3.6 ± 2.8 <sup>b</sup>	3.5 ± 3.7 <sup>b</sup>	1.6 ± 1.9 <sup>b</sup>	5.1 ± 2.3 <sup>a</sup>	4.9 ± 2.2 <sup>a</sup>	3.9 ± 2.8 <sup>b</sup>	3.8 ± 3.2 <sup>b</sup>
High protein (soy isolate)	6.7 ± 1.9 <sup>a</sup>	1.3 ± 2.4 <sup>a</sup>	2.2 ± 2.1 <sup>ac</sup>	5.9 ± 2.2 <sup>ab</sup>	5.9 ± 2.4 <sup>ab</sup>	6.5 ± 1.9 <sup>a</sup>	6.5 ± 2.4 <sup>a</sup>
High protein (egg white)	2.5 ± 2.6 <sup>b</sup>	6.3 ± 3.1 <sup>b</sup>	0.7 ± 1.3 <sup>b</sup>	5.3 ± 2.5 <sup>ab</sup>	4.9 ± 2.8 <sup>a</sup>	3.4 ± 2.8 <sup>b</sup>	2.4 ± 3.3 <sup>b</sup>
Control	6.2 ± 2.4 <sup>a</sup>	1.3 ± 1.7 <sup>a</sup>	2.6 ± 2.4 <sup>ac</sup>	6.5 ± 2.3 <sup>b</sup>	6.6 ± 1.7 <sup>b</sup>	6.3 ± 2.4 <sup>a</sup>	6.0 ± 2.8 <sup>a</sup>

<sup>a,b,c</sup>Data with different letters within the same column indicate significant difference determined by LSD post-hoc analysis (p < 0.05).

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

### 2.3. Composition of the lunch

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

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

The ad libitum test snack consisted of an assortment of different foods (sweet, salted and yogurt), generally selected by the target of subjects involved in the study: 38 g minicake (Barilla G. e R. Fratelli S.p.A, Italy), 50 g dry snack biscuits (Pavesi-Barilla G. e R. Fratelli S.p.A, Italy), 250 g low-fat red fruit yogurt (Yomo, Italy) and 60 g low-fat crackers (Barilla G. e R. Fratelli S.p.A, Italy). Subjects could eat each product as much as they liked.

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

### 2.4. Data analysis

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

The relationship among palatability-, satiety-related attributes and snack energy intake was studied by means of the Partial Least Square (PLS) regression [23]. Palatability- and satiety-related attributes scores averaged across participants were used as X matrix, whereas snack energy scores averaged across participants were set as Y matrix. Data were standardized (i.e., scaled to unit variance) prior to modeling and full cross validation was chosen as validation method. Correlation loadings plot was used to find significant variables (> 50% explained variance) [24]. PLS modeling was performed using the software The Unscrambler X (CAMO, Norway).

## 3. Results

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

### 3.1. Food palatability

Table 4 summarizes the subject's ratings related to liking and sensory properties of the 5 pasta formulations, expressed as mean ± SD. In the whole, “high fiber and high protein” and “high protein (egg white)” pasta resulted less appreciated than other formulations,

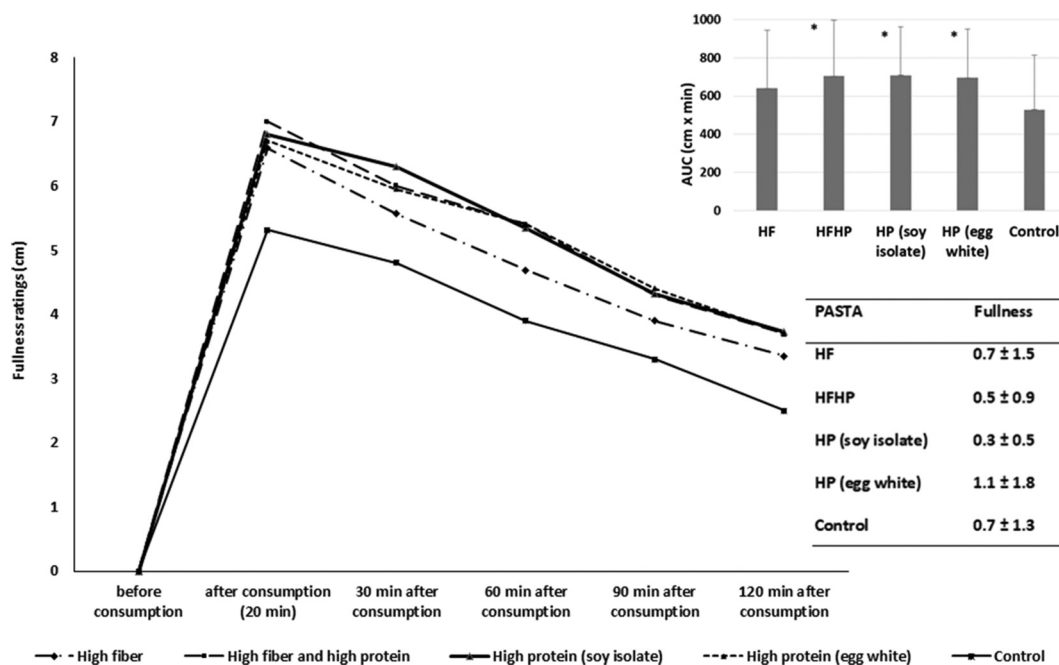


Fig. 2. Rating curves of fullness over time and related AUC values (in the box on the top right). Asterisk indicates significant differences between each formulations and control pasta ( $p < 0.05$ ). In the box on the bottom right, ratings of fullness before the lunch ( $t_0$ ) are reported. HF = “High fiber” pasta; HFHP = “High fiber and high protein” pasta; HP (soy isolate) = “High protein (soy isolate)” pasta; HP (egg white) = “High protein (egg white)” pasta; Control = Control pasta.

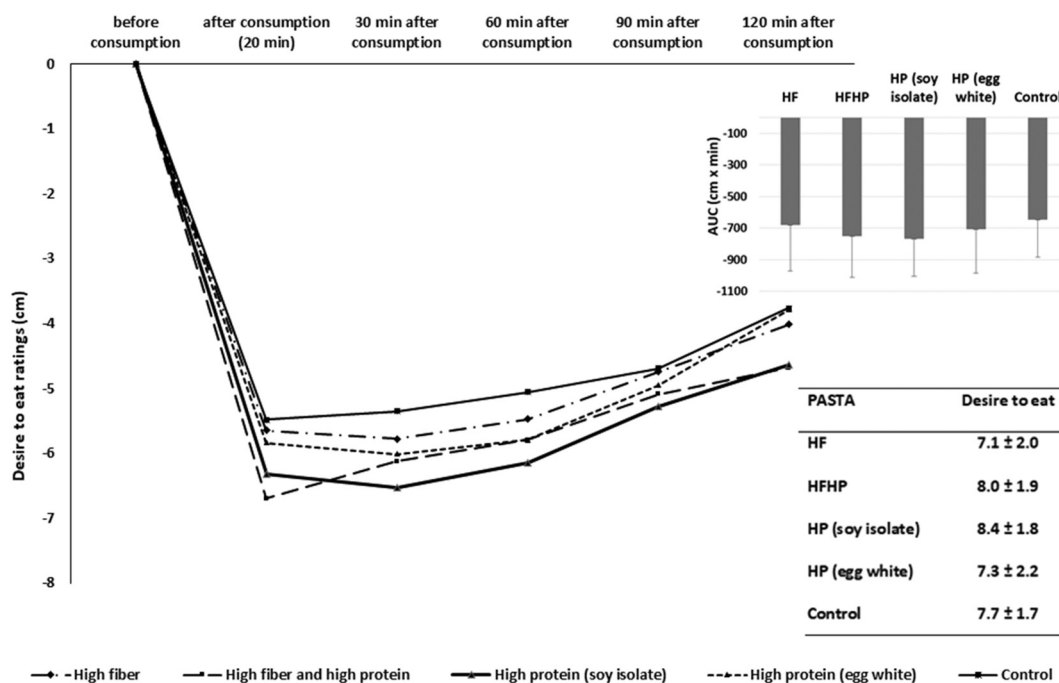


Fig. 3. Rating curves of desire to eat over time and related AUC values (in the box on the top right). In the box on the bottom right, ratings of desire to eat before the lunch ( $t_0$ ) are reported. HF = “High fiber” pasta; HFHP = “High fiber and high protein” pasta; HP (soy isolate) = “High protein (soy isolate)” pasta; HP (egg white) = “High protein (egg white)” pasta; Control = Control pasta.

receiving significantly lower scores at the questions “How pleasant is this pasta?” and “How difficult was the pasta to eat?” ( $p < 0.05$ ). The lower palatability of these formulations was due to several sensory properties, considering that texture, color and taste scores were lower than those for “high fiber”, “high protein (soy isolate)” and “control” pasta.

### 3.2. Appetite sensations after pasta consumption

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

Fullness was significantly higher in all the formulations compared to

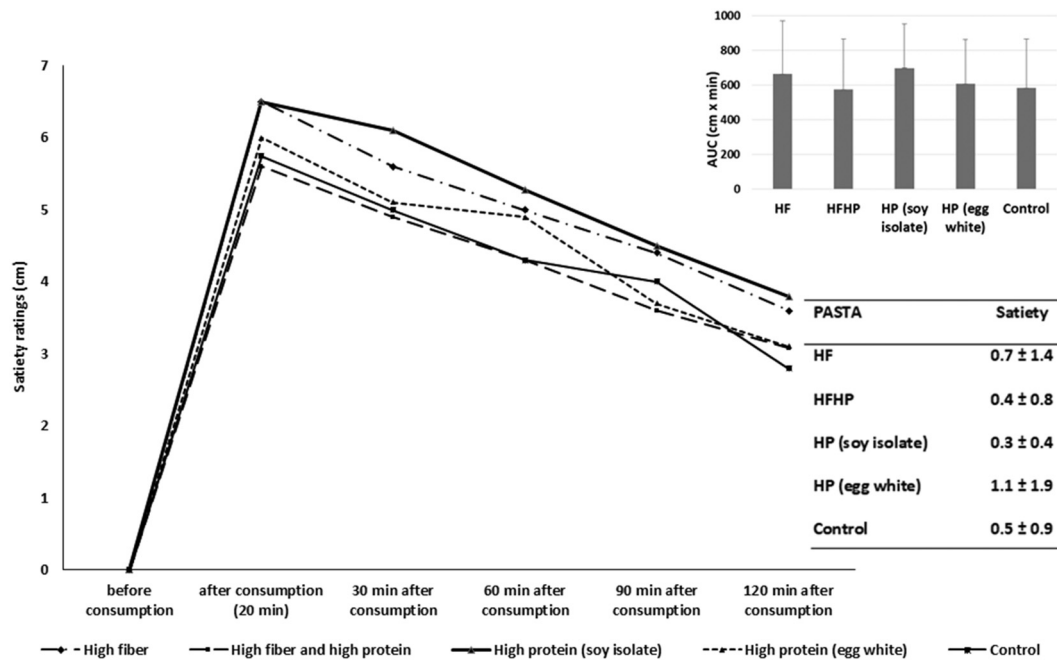


Fig. 4. Rating curves of satiety over time and related AUC values (in the box on the top right). In the box on the bottom right, ratings of satiety before the lunch (t0) are reported. HF = “High fiber” pasta; HFHP = “High fiber and high protein” pasta; HP (soy isolate) = “High protein (soy isolate)” pasta; HP (egg white) = “High protein (egg white)” pasta; Control = Control pasta.

Table 5  
Satiety quotients related to fullness, desire to eat and satiety sensations for each pasta. Values are expressed as mean ± SD.

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

<sup>a,b</sup>Data with different letters within the same column indicate significant difference determined by LSD post-hoc analysis (p < 0.05).

control pasta (p < 0.05), except for the “high fiber” pasta, and remained high up to 2 h after pasta consumption.

Overall, satiety and desire to eat were not significantly different among the five pasta formulations, even if LSD test evidenced higher satiety levels (p < 0.05) following “high fiber” and “high protein (soy isolate)” pasta compared to control, both immediately after and 2 h after pasta consumption.

On the contrary, desire to eat following “high fiber and high protein” and “high protein (soy isolate)” was significantly lower (p < 0.05) both immediately after and 2 h after pasta consumption compared to control pasta formulations.

The results obtained on individual ratings were confirmed also by the AUC analysis as shown in Figs. 1–3. In fact, it was confirmed the effect of the different pasta formulations on fullness but not on satiety

and desire to eat, with “high fiber and high protein”, “high protein (soy isolate)” and “high protein (egg white)” as the most effective formulations when compared to control pasta.

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

The quotients related to fullness resulted significantly higher for all formulations compared to control pasta both immediately after lunch and over the subsequent 2 h as indicated in Table 5. “High fiber and high protein” pasta, but not the other pasta formulations, induced significantly lower desire to eat than control pasta both after lunch and after snack consumption, while only “high fiber” pasta showed a higher satiety quotients compared to control.

### 3.3. Energy intake

Table 6 shows the energy and macronutrient intake registered after the “ad libitum” snack consumption 2 h after lunch, as well as the amount of each food consumed. Statistical analysis shows that only “high fiber and high protein” formulation significantly influenced the energy intake at snack-time if compared to the other formulations.

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

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

In the Scores plot (Fig. 5A), pasta formulations near to each other are considered as similar, while pasta formulations positioned far from each other are different for the selected variables. Similarly, in the Correlation loading plot, variables laying near the Y variable (Energy, indicated in red in Fig. 5B) give a positive contribution to the Y estimate, while variables located in the opposite part of the graph contribute negatively to the Y estimate.

The Correlation loadings plot shows that Palatability-related attributes (except difficulty to eat) are positively correlated to snack energy

**Table 6**  
Energy and macronutrient intake through snacks consumed 2 h after pasta consumption (n = 19). Values are expressed as mean ± SD.

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 yogurt	Water				
HF	231 ± 67 <sup>b</sup>	7.23 ± 3.0	2.8 ± 1.5	0.8 ± 0.4	42.9 ± 13.2	24.5 ± 12.7	1.6 ± 0.8	13.7 ± 18.2	12.2 ± 14.4	15.8 ± 17.9	99.9 ± 83.7	228 ± 67				
HFHP	219 ± 93 <sup>a</sup>	7.5 ± 3.0	2.4 ± 2.0	0.7 ± 0.7	40.4 ± 17.4	25.2 ± 12.2	1.4 ± 0.8	12.9 ± 22.5	10.3 ± 11.6	10.8 ± 14.9	123.8 ± 80.2	235 ± 172				
HP (soy isolate)	263 ± 91 <sup>b</sup>	8.4 ± 3.8	3.5 ± 1.6	1.1 ± 0.5	48.0 ± 12.7	26.5 ± 13.6	1.9 ± 0.8 <sup>a</sup>	21.1 ± 19.4	14.3 ± 14.7	13.0 ± 14.7	113.8 ± 87.5	236 ± 154				
HP (egg white)	234 ± 96 <sup>b</sup>	7.5 ± 3.2	2.9 ± 2.1	0.9 ± 0.7	43.2 ± 18.2	25.4 ± 13.9	1.6 ± 0.9	16.9 ± 26.8	10.8 ± 15.0	13.7 ± 17.0	109.4 ± 73.3	232 ± 132				
Control	267 ± 106 <sup>b</sup>	8.6 ± 3.9	3.6 ± 2.1	1.1 ± 0.7	48.7 ± 19.1	26.9 ± 14.0	1.9 ± 1.1	21.4 ± 23.1	15.0 ± 18.2	12.0 ± 17.2	119.8 ± 78.6	223 ± 145				

HF = “High fiber” pasta; HFHP = “High fiber and high protein” pasta; HP = “High protein (soy isolate)” pasta; HP = “High protein (egg white)” pasta; Control = “Control” pasta.

<sup>a,b</sup>Data with different letters within the same column indicate significant difference determined by LSD post-hoc analysis (p < 0.05).

intake, whereas fullness variation and AUC as well as difficulty to eat are negatively correlated to snack energy intake. The Correlation loadings plot also shows that satiety and desire to eat (both variation and AUC) play a marginal role in predicting snack energy intake.

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

#### 4. Discussion

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

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

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

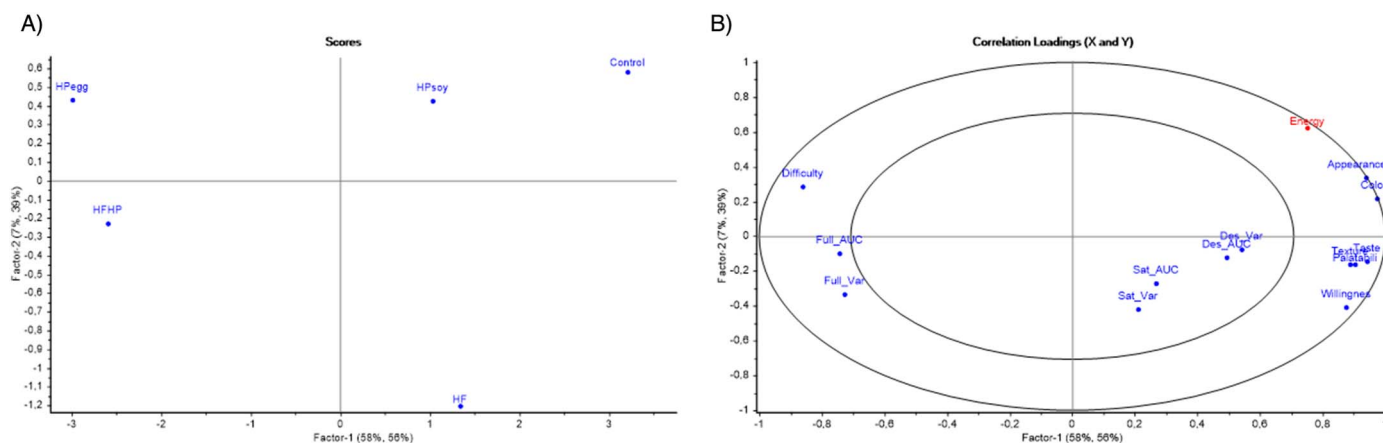
All the pasta formulations compared to the control pasta significantly affected satiety-related sensations; however, the differences in palatability among products seem to be an important modulatory factor.

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

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

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

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



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

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

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

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

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

In the present study, we also considered the satiety quotients, introduced by Green et al. [22] to standardize the satiety sensations in relation to the energy intake. Satiety quotients can give a measure of the extent to which the food eaten during the eating episode reduced subjective appetite per unit of intake. Our results on satiety quotients did not differ from those on satiety-sensation ratings and AUC. As an exception, quotient for satiety and fullness following “high fiber” pasta were significantly lower probably due to the lower energy content compared to the other formulations.

As regards mid-afternoon snacking, we found that only the “high fiber and high protein” pasta formulation had a significant effect on subsequent energy intake. These results are partially in contrast with those reported by the above-mentioned study by Korczak et al. [39], who did not find differences among the pasta treatments for snacking. In that study, as discussed by the authors, since the majority of subjects were students with limited budget, they ended up consuming most of

the snacks regardless of any satiety-related sensations. In addition, differently from our study, the blend of snacks included highly palatable and appealing foods, thus probably contributing to the failure in the control of food intake. Finally, differences among findings can be ascribed to the different composition of pasta formulations as well as to the fact that our study was performed in a group of Italian volunteers for whom pasta is consumed on a daily basis.

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

## 5. Conclusions

In the present study, we investigated the effect of consumption of five different pasta formulations made with protein from different sources, alone or in combination with fiber, on satiety response and energy intake in young healthy women. Our results and those from the literature reveal that many factors must be taken into consideration to better detail the effect of a food on satiety and eating behavior including cultural and attitudinal characteristics. In particular, from our results new pasta formulations with combinations of fiber and protein seem to be effective in the modulation of appetite sensations and subsequent energy intake, at least in our condition of preparation and specific target population (i.e. young women). In this regard, it is important to consider that sensory properties of foods seem to be critical determinants of eating behavior, thus a fundamental variable for the design of products tailored to different types of consumers.

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