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Research Report

# Can a different pasta making process preserve the starch's ultrastructure, increasing its digestibility?

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**Abstract**.
**BACKGROUND:** Pasta plays an important role in human nutrition, nevertheless its organoleptic, structural and bromatologic properties have not been completely studied.
**OBJECTIVE:** The study aims to compare the ultrastructure pasta cross-sections produced with reduced mechanic stress and low heat exposure technique (Pietro Massi Technology ™) to common samples obtained with traditional technology in order to assess if a different process can alter the ultrastructure of starch and then, its digestibility.
**METHODS:** A comparative study was performed on the ultrastructure of pasta using scanning electron microscopy (S.E.M). 55 were pasta cross-sections made with Pietro Massi technology, 33 were samples from different pasta factory produced using traditional process, for a total of 88 samples analysed.
**RESULTS:** According to an Index based on what it was observed at one slide at S.E.M we classified samples and compared the number of grains, caves and canals visible, their dimensions in μm and average size of grains. The number of grains in group 1 ($9.06 \pm 5.01$) in group 2 ($5.71 \pm 5.77$), in group 3 ($5.93 \pm 7.65$) resulted significative more elevated compared to group 4 ($0.73 \pm 2.58$); in group 1 and group 2 the number of caves observed was higher than group 4. The size in μm of canals resulted significantly more elevated in group 1 than group 3 ($p = 0.008$), group 2($p = 0.013$), and group 4 ($p = 0.02$).
**CONCLUSIONS:** The analysis of average size of caves and canals in μm, the number of caves and grains demonstrated in samples obtained with Massi technology elevated values compared to samples obtained with traditional technique. The number of caves, grains and canals visible in the starch surface demonstrate the high quality of this pasta because they likely ensure penetration of water during cooking, facilitating at the same time the penetration of pancreatic amylases during digestion, suggesting a better digestibility in pasta produced according to this methodology.

Keywords: Index, starch, quality, technology, food-production

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## 1. Introduction

After bread, pasta is the second most consumed food product in the world [1] playing an important role in human nutrition. Pasta has indeed a low Glycaemic Index(GI) and in the context of low-GI dietary patterns, it has been demonstrated that could reduce body weight and Body Mass Index (BMI) compared with higher-GI dietary patterns [2]. Although pasta is considered a refined carbohydrate with a very simple composition, made only by water and durum wheat that can be shaped differently [3], its organoleptic, structural and bromatologic properties have not been completely studied. Traditionally pasta is made exclusively with durum wheat flour, but often soft wheat and/or other cereal flours are added [4]. Some authors [5] suggest the use of pasta enriched with faba, bean or peas flour to increase and complete its natural nutritional power [6–9].

The quality of the raw material used [10, 11] (i.e. genetic stream of the wheat used [12], the cultivation or harvesting methods), how semolina is processed and how the product is conserved (pasta can be cooked fresh or stored after drying) can improve the final pasta quality. The way wheat is processed and the flour is kneaded in a dough are the phases that mainly impact on the final product [4, 13].Conventionally pasta is produced by a rapid extrusion of a durum wheat semolina dough through dies, followed by a drying stage in strictly controlled conditions (dried pasta) [14].

Manufacturing process, particularly heat and mechanic stress, can modify both organoleptic and nutritional pasta properties. Khatkars and Watanabe have already proved the role of ultrastructure analysis in defining feature and quality of dough according to the type of process and the composition in gliadin and other components [15, 16]. Nevertheless, there are not comparative studies on the different process making of pasta and further, scanning electron microscopy (S.E.M.) and other tools are not used and validated to assess the quality check of pasta or its nutritional properties. Reserches did not analysis ultrastructure of stored pasta after drying and, above all, on how the pasta is processed.

In order to assess whether the different phases of pasta production could affect the final results, we performed a comparative study on the ultrastructure of the pasta visible with S.E.M., comparing pasta obtained with an innovative technology that should preserve the ultrastructure of starch from heat and pressure to cross-sections obtained with traditional technology. The *primus movens* was represented by the evidence that a preserved starch ultrastructure might increase digestibility of pasta as catalytic enzyme can have a facilitated access to grains of starch.

## 2. Material and methods

### 2.1. Study design

The study aims to compare the ultrastructure of pasta cross-sections produced with technique that reduce the mechanic stress and heat exposure (PMT: Pietro Massi Technology™) to common samples obtained with traditional technology (TT), in order to assess if a different process can alter the ultrastructure of starch and then, its digestibility. The operators, responsible for ultrastructure analysis, identified samples by anonymous code.

### 2.2. Pasta samples

Samples produced with PMT were provided by manufacturer and chosen randomly between those destined to market. Cross-sections from other pasta factory were purchased in shops and chosen between more popular brands suggested by retailers. In either case, we verified the integrity of pasta package and the expiry date. Samples were then preserved according to the producer's indication in the label.

Eighty-eight samples were analysed: 55 were made with PMT, 33 were samples from TT. We analysed both long and short pasta. The cross-sections were made with different wheat and doughs (durum wheat, wheat plus albumen or wheat plus eggs) as described in Table 1.

Table 1
Samples were divided in two groups: samples made with PMT and samples made with TT. For each group, it is specified the raw used

| Type of technology | Bran | | Bran plus eggs | | Albumen | | Total | |
|---|---|---|---|---|---|---|---|---|
| PMT | 17 | (19.32%) | 34 | (38.64%) | 4 | (4.54%) | 55 | (62.5%) |
| TT | 15 | (17.04%) | 15 | (17.04%) | 3 | (3.41%) | 33 | (37.5%) |
| Total | 32 | (36.36%) | 49 | (55.68%) | 7 | (7.95%) | 88 | (100%) |

Presently, Italiana Pastifici holds the Pietro Massi 's patents[1]. The PMT is the result of a reduction in the number of components normally present in conventional production line in order to prevent the mechanical stress. Mechanical stress can indeed determine rise in temperature and alter properties of raw materials. The PMT is cold throughout, without attrition, shear stress or alterations in temperature that could compromise the final product. The process takes place in four steps:

1. Moisturising, pre-dough and dough.
2. Rigorously cold rolling for long pasta (LAR System). Rolling for long pasta is based on the use of particular stainless steel rollers capable of creating a pasta sheet from dough crumbs in a single step, using a cold mono-process at a constant temperature which completely eliminates the kneading process.
3. Drawing and die-cutting of the dough for short pasta. For the short pasta, the new system of drawing and die-cutting the dough means that it can be made into any shape, using the cold technology process. It is based on a system of gradual compression, which eliminates areas of possible inactivity, thus preventing contamination or changes to the organoleptic qualities of the product.
4. Pre-drying and drying.

### *2.3. Scanning electron microscopy*

The analysis of ultrastructural characteristic was based on the observation of pictures obtained with S.E.M. (NovaNano SEM 450 Bruker E. FF.) in the laboratory of Modena e Reggio Emilia, in the University of Rome (La Sapienza) and University of Parma (Dipartimento di Scienze della Terra).

The cross-sections were examined at different magnification, in analysis of surface and/or after cutting, without any manipulation which could alter the structure. The average mag X used was $1287.6 \pm 700.25$; the spot size medium was $26.34 \pm 76.88$; the working distance mean used was $8.02 \pm 2.39$. Two investigators in double blind examined the structure of starch at S.E.M.

### *2.4. Classification*

We adopted the following criteria in order to classify pictures, considering the chemical integrity of starch, its visibility at S.E.M., its surface (rough or not). According to this Quality Index (Q.I.), we divided cross-sections in four classes:

*Group 1*: the analysis by S.E.M show perfectly intact starch and rough and solid surface;

*Group 2*: the starch was intact, the surface was not flat;

*Group 3*: flat starch not really viewable;

*Group 4*: the starch is totally damaged and its surface is not assessable.

[1] n. 2 patents for the production and lamination of pasta IT/EU n. 0001345718 and IT/EU n. 0001364237; n. 2 patents for the drawing and modelling of tagliatelle IT/EU n. 0001365805 and IT/EU n.0001379743 n. 1 patent for the dough-making system IT/EU n. 0001409093

### 2.5. Statistical analysis

Continuous variables were expressed through means and standard deviation, while dichotomous variables were expressed like number and percentage of samples. The Student's *t*-test was used to compare normally distributed continuous variables. ANOVA test was used to compare means and standard deviation between different groups. The results were considered statistically significant for values of $p < 0.05$.

## 3. Results

Cross-sections tested were:

- puff pastry (Italian tagliatelle) with eggs, 16(18.18%). 15 were produced with LAR lamination technology, 2 with extrusion printing press. About the 15 samples produced with LAR technology: 7 of them were made with lamination in one step; 2 samples were made with lamination of 350 diameter in one step,1 lamination diameter 350 plus calibration/refining. 1 of lamination 500 diameter in one-step,1 lamination diameter 500 plus calibration/refining,1 lamination diameter 700 one step, one lamination diameter 700 plus calibrating/refining.
- long pasta, 16(18.18%). 7 were made of CWAD (Canadian Western Amber Durum)bran and egg, 5 only with egg, 4 with hulled wheat plus eggs.
- half maniche, 25(28.41%): 17 made with bran without eggs, 4 only eggs, 4 only albumen. Half maniche were produced by extruding process.
- strozzapreti were 13(14.87% of the whole), made only with eggs.
- in addition there were 9 samples of half rigatoni with only bran, 3 rigatoni only semola, 3 samples of bio fusilli and 3 tagliardi with albumen.

When we focused on the technology used for making pasta, the 88 samples were divided in two groups: 55 were samples of pasta made with PMT, 33 were samples from TT.

For each group, we considered the raw used (Table 1).

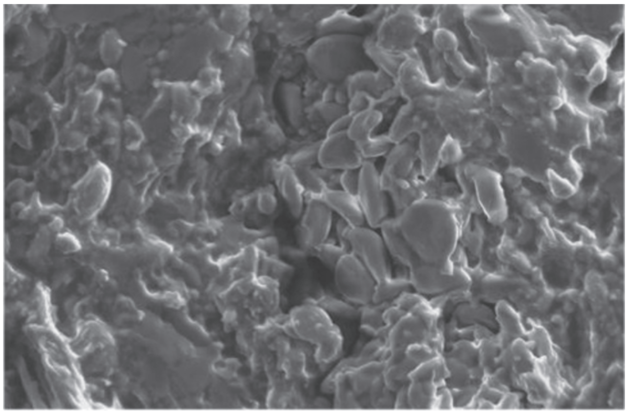
Table 2 shows the number of types of pasta analysed via S.E.M. belonging to the different groups as per the Q.I. classification's criteria. No PMT pasta types belonged to Group 4, whist the 45.45% of TT pasta's types belonged to this group (Table 2).

The examination with S.E.M. showed different features of starch: its surface; number and size of caves, canals and visible grains. The picture appended [1–6] are some examples of what can been seen at S.E.M, divided for class according to the Q.I.. More picture can be provided on reasonable requests.

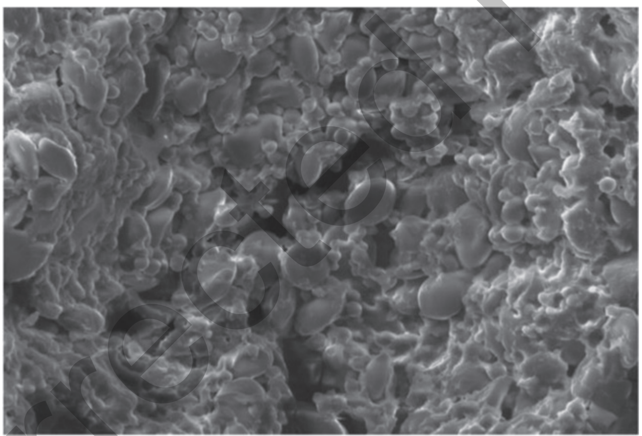
As reported in Table 3, the average size of caves and canals in μm was higher in samples obtained with PMT. Also, the number of caves and grains was significantly higher in sample by PMT than samples from TT.

Table 2
According to Q.I. it was reported the number of samples for each group, distinguished in base of different technology used

| Groups | PMT | TT |
|---|---|---|
| 1 | 17 | 0 |
| 2 | 25 | 3 |
| 3 | 13 | 15 |
| 4 | 0 | 15 |

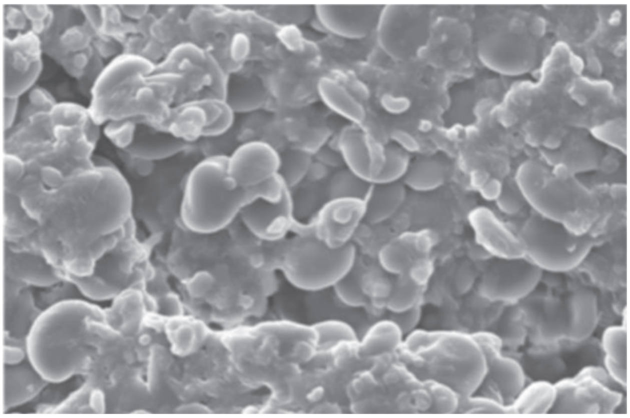


Picture 1. Example from Class 1 according to the Q.I.. Tagliatelle made with eggs produced with PMT at 1500 kV analysed at Sapienza University. The picture show several grains of starch perfectly intact. The starch don't suffer any damage during lamination 350 and refinement. Independently from the type of lamination used, the starch is perfect and caves, where the water can distribute, are present. Mag X 2000; brightness 49.7%; spot size 4.0; WD 6.3mm.



Picture 2. Example from Class 1. Tagliatelle with eggs obtained from PMT examined at Sapienza University at 1500 kV.The picture show grains of starch perfectly intact. The starch don't suffer any damage during lamination 700 and refinement. Independently from LAR used, the starch is perfect, solid and rough, caves are present. No mechanic stress during the phase of lamination. Otherwise the major compression might have destroyed starch with more water relapse during cooking. Mag X 2000; brightness 49.5%; spot size 4; WD 7.2 mm.

We used Q.I. to compare with ANOVA the number of grains, caves and canals which were visible at S.E.M., their dimensions in μm and average size of grains. The number of grains observed at S.E.M. was higher in group 1 than the group 4 ($p < 0.001$) but also the number of grains in class 2 and 3 was elevated compared to class 4 ($p < 0.008$). There were no difference between groups 1, 2 and 3. The average size of grains was not different between the four classes. The number of caves was higher in group 1($p < 0.002$) and group 2($p < 0.06$) than in group 4. The group 2 had an average size of caves higher than group 4 but there was not significant difference between groups 3 and 2. The size in μm of canals resulted significantly more elevated in group 1 than group 3 ($p < 0.008$), group 2($p < 0.013$), and group 4 ($p < 0.02$).



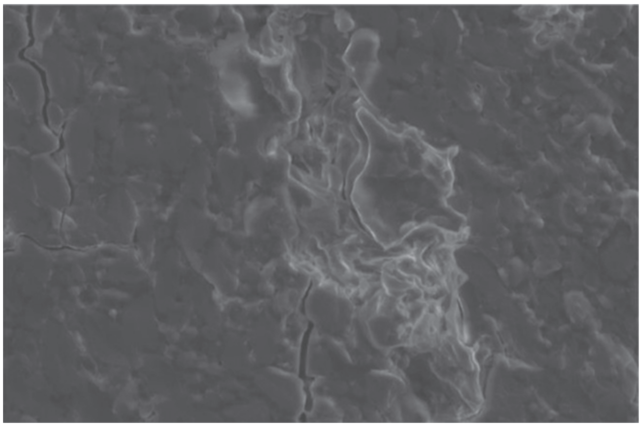
Picture 3. Example from Class 2. Tagliatelle with eggs obtained with 350 lamination analysed at Sapienza University at 1500 kV. The surface is rough, solid, compact. There are caves and grains. This sample came from Italiana Pastifici which uses Massi's technology. Mag X 1500; spot size 288; WD 10 mm; brightness 49.7%; contrast 33%; EHT 2000kV; Fil I 2.717 A.

Picture 4. Example from Class 3. Strozzapreti made in TT with eggs. Analysed by Modena at 2000 kV. Starch is not well displayed. Surface is flat. Grains are not uniform. Mag X 500; spot size 4; WD 12.6.

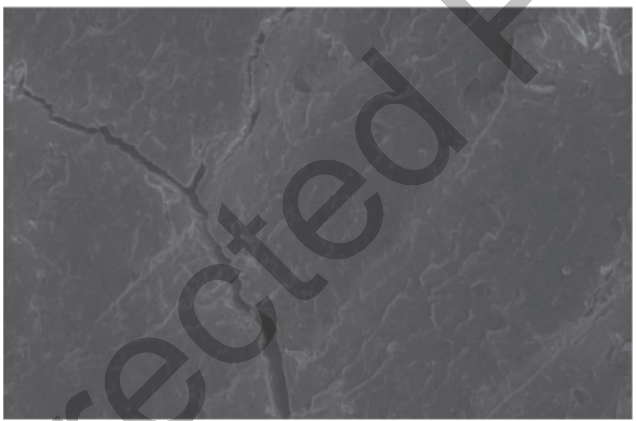
Table 3

Always using the division in two groups (PMT vs no TT) it was calculated the mean and standard deviation of number of grains and their mean and s.d. size. We also consider the major size in of canals and caves in μm for each group. Then, we assess the average number of canals and caves visible at SEM. The results were considered statistically significant for values of $p < 0.05$

| | PMT | TT | *P* value |
|---|---|---|---|
| Mean and s.d. size of grains | 13.95 ± 6.21 | 15.3 ± 6.52 | 0.57 |
| Mean and s.d. caves in μm max | 20.25 ± 21.96 | 7.08 ± 13.74 | **0.01** |
| Mean and s.d. canals in μm max | 3.27 ± 7.01 | 0.73 ± 1.42 | **0,05** |
| Caves number samples | 1.54 ± 1.57 | 0.64 ± 0.89 | **0.04** |
| Canals number samples | 1.24 ± 1.59 | 0.52 ± 0.87 | 0.06 |
| Mean number of grains visible | 7.33 ± 6.18 | 2.67 ± 5.64 | **0.001** |



Picture 5. Example from Class 4. Rigatoni analyse by University of Modena at 1500. The surface is flat and damaged, there is only one caves. Holes and cracks are present. Mag X 2000; spot size 4; WD 7.3.



Picture 6. Example from Class 4. Half rigatoni analysed by University of Modena at 1500kV. Surface is completely destroyed and a crack divides in two the starch. The starch is stretched and pressed by mechanic stress. Mag X 1000; spot size 4; WD 5.9 mm.

Table 4
Mean and standard deviation of size of grains, caves and canals; mean and s.d. of number of grains, caves and canals observable at S.E.M

| Class | Mean and s.d. N. grains | Mean and s.d. size grains | Mean and s.d. N. Caves | Mean and s.d. Caves in µm | Mean and s.d. N canals | Mean and s.d. canals in µm |
|---|---|---|---|---|---|---|
| 1 | $9.06 \pm 5.01$ | $13.5 \pm 6.78$ | $1.93 \pm 2.25$ | $17.47 \pm 18.48$ | $0.64 \pm 0.63$ | $6.73 \pm 10.98$ |
| 2 | $5.71 \pm 5.77$ | $15.26 \pm 5.98$ | $1.56 \pm 1.23$ | $19.9 \pm 21.93$ | $1.13 \pm 1.85$ | $1.27 \pm 2.72$ |
| 3 | $5.93 \pm 7.65$ | $14.00 \pm 6.46$ | $1.00 \pm 0.94$ | $19.16 \pm 23.57$ | $0.5 \pm 0.89$ | $0.95 \pm 1.73$ |
| 4 | $0.73 \pm 2.58$ | $11.00 \pm 2.83$ | $0.13 \pm 0.35$ | $2.07 \pm 5.71$ | $0.8 \pm 1.01$ | $1.07 \pm 1.67$ |

The mean and standard deviation of number of caves, canals and grains for each group obtained with Q.I. is reported in Table 4.

## 4. Discussion

To assess quality of traditional pasta, selection of raw is crucial [10] as well as the method of conservation and the production process [17, 18]. Indeed, the level of hydration, the different temperature [19] and the mechanical forces play a role in changing starch conformation and this can alter the unstable balance of its molecular interaction [20], modifying starch digestibility [21]. For instance, the starch hydration can affect the efficiency of enzymes [22, 23] as amylase affinity and catalytic efficiency is reduced with a low water content [24]. Humidity or heat, produced by pasta machine during manufacturing process can destroy starch surface [10] and decrease its digestibility due the high-temperature drying process, determining at the same time proteins aggregations through cross-link, via covalent bonds, and disulfide bridges [25]. Higher pressure levels can disrupt the starch granule morphology and induce the starch gelatinization [26]. Digestibility decreases because of extrusion that creates starch-protein and starch-lipid complexes [27]. When the starch is destroyed and its surface is flat, the consequence is that nutritional components are totally or partially released in the boiling water. Moreover, gelatinisation and retrogradation, which correspond to structural modifications in the granules and affect the behaviour of starch-containing systems, result distorted. In the event that the surface is flat and altered, gelatinisation and retrogradation do not start from intact, perfect starch but from a polymeric matrix of glucose with few gliadin bridge and without superstructure [28, 20]. The three-dimensional crystal-like starch structure with double stranded amylopectin alternated to amorphous area is essential in its complexity in all the steps of pasta production [29, 30], from cooking to storage and from taste to digestion (hydrolysis and proteolysis).These are some of the reasons that led us to consider the integrity of starch surface as main factor in establishment of possible pasta Q.I. High quality pasta has to be easily digested: hydration, mechanical forces and temperature contribute to maintain a perfect ultrastructure of starch which influence eventually the digestibility.

Currently, what a pasta Q.I. is, is still under debate: cooking performances and nutritional/organoleptic characteristics are the aspects commonly considered in assessing pasta quality [31]. The relish of North-American consumer and the cooking performance are very different from European one and a scientific approach is quite impossible, because reference parameters depends on the reference market. Although some results are reported in the literature [32] those are all based on either sensorial measurements or empiric ones. Therefore, they cannot be used in a scientific approach or in a predictive model. Furthermore, the nutritional/organoleptic macro-category are directly related to Maillard reaction, a set of chemical reactions [33].

In literature there are not reported indexes for pasta based on observation of one slide at S.E.M. validated internationally. For this reason, we made samples classification considering the starch importance as pasta component. The idea was to create a simple index, using imagine from S.E.M, which can be understood also by no experts and by the normal consumer, to make them capable to see and therefore understand what they are eating. We were pushed by philosophical principle "We are what we eat" of Feuerbach and without doubt, a perfect intact surface is more appetizing than a damaged one. A Chinese proverb affirms that a picture is worth more than 10,000 words. This proverb gave the push for a study and comparison of 54 different type of starch [34], where the pictures are the one produced via S.E.M.

As long as starch is what make high quality pasta, we focus then on its structure as it is visible at S.E.M.. We choose S.E.M. because it has some major advantages over light microscopy: it has hundreds of times greater depth of focus than the light microscope, and, above all, it has a much higher order of resolution and magnification. This instrument permits the study of the faint surface starch structure.

The first thing observable at S.E.M. is the presence of starch granules: small spherical B-type granules (average diameter 23 mm) and larger lenticular A-type granules (average diameter 30 mm) [35]. The size and distribution of starch granules are extremely important to discover functional and physico-chemical properties of pasta starch.

Starch granule size can affect the elastic modulus and mixing time of pasta and the rheological properties of wheat flour. Increased proportions of smaller size granules increase the dough's elastic character [36]. Then, we speculated that the presence of more, smaller granules can increase enzymes catalytic affinity to starch, improving digestibility. In our study, samples from pasta PTM have a significative increased number of grains compared to sample produced with TT.

The second point is the presence of canals and caves which allows the penetration of water into pasta during boiling. A major hydration can positively influence amylase activity, making starch more attachable from pancreatic enzyme and facilitating digestion in the intestine. The surface canals and interior caves are believed to be naturally occurring features of the starch granule structure [37]. Compared to TT, pasta produced with PMT demonstrated average size in μm of caves and canals significatively increased. Furthermore, the number of caves was significantly higher in sample by PMT than samples from TT.

The presence of hole and cracks was considered as a negative quality of starch due irregular and heterogeneous surface. They may have been due to shrinkage during sample preparation or tension within the pasta dough during drying. Also Cunin, et al. [38] and Dexter et al. [39] have already observed the phenomenon: durum wheat semolina pasta dried at an ambient temperature (22–25°C) with 30% humidity will crack and the strands broke into small pieces. The strands could not hold their shape: this indicates that the pasta drying process plays an important role in starch integrity [40]. We speculate that the best results reported by PMT derives from the use of its cold technology, innovative in each phase of process, standing out from traditional methods from the hydration to pre-dough and dough and from lamination to pre-drying and drying.

In conclusion, as pasta is one of most common food in all the world, we focus on it for its clinical relevance in determining GI but also for the close relationship between food, immune response and individual antiviral defence [41] which needs to be better investigate. Probably the characteristics of the final mixture (starch and protein component) and in particular its rheological characteristics (viscosity, elasticity, toughness, etc.) can influence GI. GI measures the relative glucose-raising property of carbohydrates containing foods compared to glucose or white bread with equivalent amount of carbohydrates [42]. The preserved ultrastructure of pasta can justify slower digestion and delayed gastric emptying which are the foundations of low GI. Pasta indeed with its low GI could reduce body weight and Body Mass Index. As obesity is one of the most important risk factor for diabetes mellitus 2, we speculate that pasta may have a positive effect on long-term cardio-metabolic disease risk. Further studies should be conducted to understand first how starch can be better processed, focusing on how processing can influence its ultrastructure and then, its digestibility (PMT in fact demonstrated a good relationship with the integrity of ultrastructure), secondly how pasta can be digested in the bowel and which are the qualities of the best starch in order to be easily digested, preserving the taste which make pasta the most loved food.

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## Ethics approval

All the procedures performed in this study were in accordance with the ethical standards of the institutional or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

## Datasets

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declaration of Conflicting interests

G. Castiglione works for Italiana Pastifici SrL, Imola. The other authors declare that they have no conflict of interest.

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