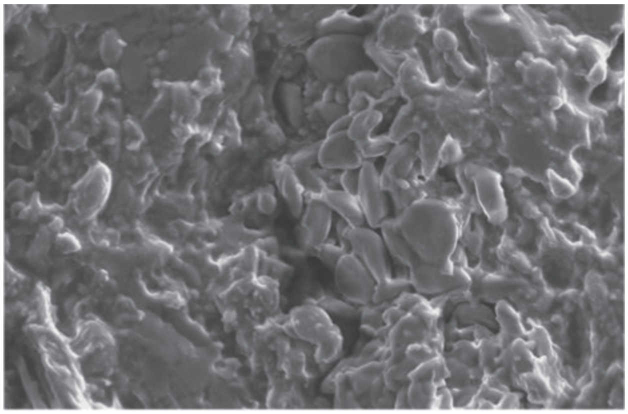
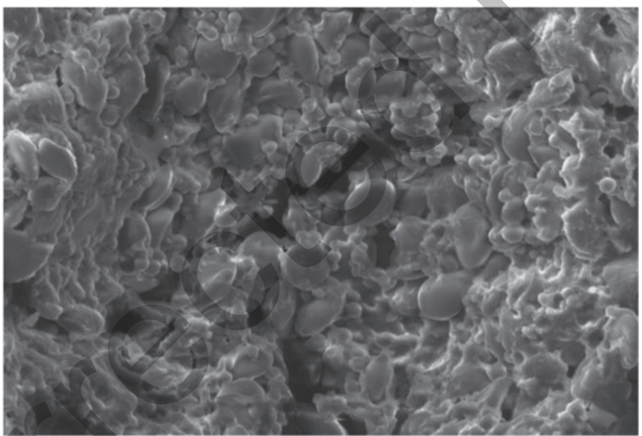


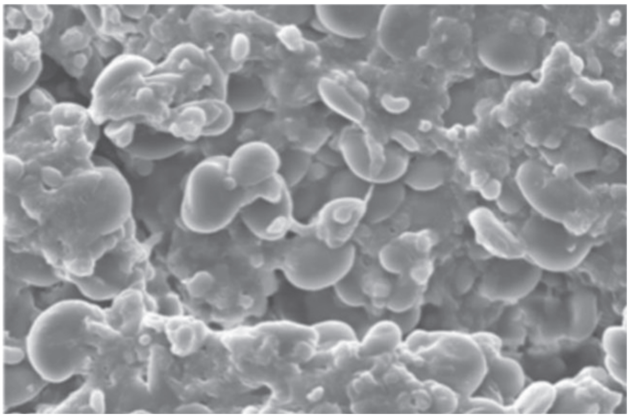
Picture 1. Example from Class 1 according to the Q.I.. Tagliatelle made with eggs produced with PMT at 1500 kV analysed at Sapienza University. The picture show several grains of starch perfectly intact. The starch don't suffer any damage during lamination 350 and refinement. Independently from the type 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 ($p < 0.02$).

Corrected Proof



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.

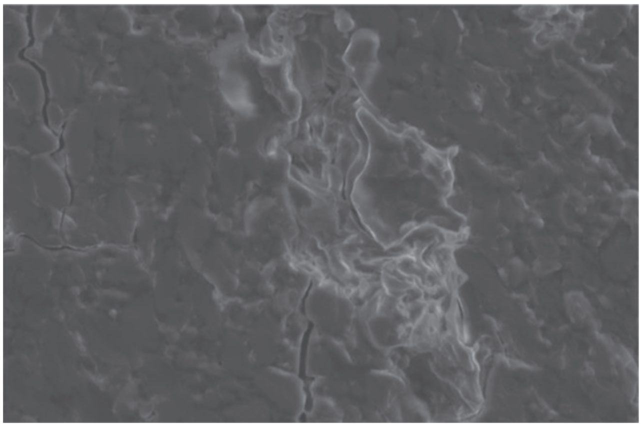
Picture 4. Example from Class 3. Strozzapreti made in TT with eggs. Analysed by Modena at 2000 kV. Starch is not well displayed. Surface is flat. Grains are not uniform. Mag X 500; spot size 4; WD 12.6.

Table 3

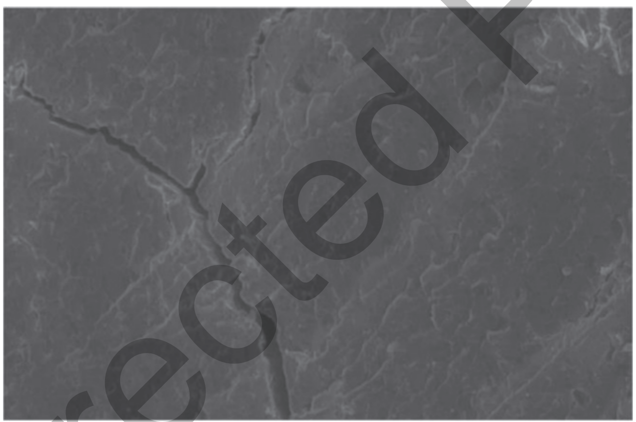
Always using the division in two groups (PMT vs no TT) it was calculated the mean and standard deviation of number of grains and their mean and s.d. size. We also consider the major size in of canals and caves in μm for each group. Then, we assess the average number of canals and caves visible at SEM. 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**

Corrected Proof



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 ± 5.01	13.5 ± 6.78	1.93 ± 2.25	17.47 ± 18.48	0.64 ± 0.63	6.73 ± 10.98
2	5.71 ± 5.77	15.26 ± 5.98	1.56 ± 1.23	19.9 ± 21.93	1.13 ± 1.85	1.27 ± 2.72
3	5.93 ± 7.65	14.00 ± 6.46	1.00 ± 0.94	19.16 ± 23.57	0.5 ± 0.89	0.95 ± 1.73
4	0.73 ± 2.58	11.00 ± 2.83	0.13 ± 0.35	2.07 ± 5.71	0.8 ± 1.01	1.07 ± 1.67

Corrected Proof

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.

Corrected Proof

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.

Corrected Proof

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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