Ultrastructure of pasta from gluten-free grains: a brief overview

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SUMMARY

In dry pasta from durum wheat the protein component, namely gluten, plays a key role in determining its quality. The organization of proteins in a dense and structuring gluten network is crucial for keeping starch granules within the matrix and thus limiting the release of organic material into the cooking water. Good development of gluten-network is also associated with low stickiness and firmness of cooked pasta. In pasta from gluten-free (GF) grains, the structuring role of gluten can be undertaken by the formation of a scaffold of retrograded starch or by other proteins intentionally added into the formulation. However, the production of good quality GF-pasta (*i.e.*, high firmness, low stickiness, and cooking loss) still represents a challenge for the food industry. The use of microscopy to better understand the ultrastructural organization of single components, either in conventional or GF-pasta, represents a powerful and informative tool of investigation. Different microscopy techniques can be specifically applied to study pasta structure as affected by processing or by different raw materials. In this review article, the impact/effect of processing conditions, as well as the use of the most popular GF-grains in the production of GF-pasta and their positive and negative implications on the ultrastructure of the final product are presented.

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Introduction

The popularity of pasta and its consumption are constantly increasing worldwide thanks to several reasons, including its taste, versatility, as well as nutritional properties and reasonable price (Dello Russo *et al*., 2021). Durum wheat semolina is the preferred raw material for pasta production: high protein content and tenacious gluten are crucial characteristics to obtain pasta with "al dente" texture and optimal cooking performance (Marti and Pagani, 2013). When observed at the confocal laser scanning microscopy (Figure 1), developed gluten appears like a dense and tick network of protein filaments that serves to support the scaffolding of the future product and to retain other flour components like starch granules (Cardone *et al*., 2020). The progressive formation of intermolecular disulphide (SS) bonds by oxidation of SH-groups of cysteine residues and/or SH-SS interchange reactions brings to the formation of a gluten network of increasing strength. Indeed, gluten network is responsible for the cohesiveness required for making pasta and for limiting the swelling of starch granules, thus ensuring a firm consistency and low stickiness in cooked pasta (Marti *et al*., 2016). For durum wheat pasta, good cooking quality is strongly related to a compact structure characterized by swollen and gelatinized starch granules trapped inside the continuous network formed by coagulated gluten proteins (Cunin *et al*., 1995; Marti *et al*., 2014a). Creating this kind of structure starting from non-gluten raw materials is quite challenging but necessary**,** taking into consideration the increasing percentage of consumers eating gluten-free (GF) pasta. Gluten replacement in pasta is one of the major technological challenges that the food industry has been facing in the last 15 years and a variety of ingredients and/or processing conditions have been so far proposed to produce pasta from GF raw materials (Marti and Pagani, 2013). The aim of this review is to describe the ultrastructure of GF pasta as affected by processing conditions or ingredients and to relate it to the cooking behaviour of the final product.

Ultrastructure of GF pasta as affected by processing conditions

Starch, and specifically retrograded starch, is the main structuring component in GF pasta (Marti *et al*., 2010; Mariotti *et al*., 2011) and various processing methods have been proposed to produce GF pasta (Marti and Pagani, 2013). The first approach - namely extrusion-cooking - consists in treating a native GF flour with steam followed by an extrusion at high temperatures (>100°C) to promote starch gelatinization inside the extruder-cooker. After its gelatinization, starch will undergo a re-organization (i.e., retrogradation) creating a structure able to withstand cooking. The second approach is based on the use of pre-gelatinized GF flour which is formed into pasta using a continuous press as for semolina pasta production.

Marti and Pagani (2013) highlighted differences in starch arrangement inside rice pasta produced using the above-mentioned technologies. Pasta from 100% pregelatinized flour was characterized by compact and homogeneous matrix, whereas in pasta from the extrusion-cooking of native rice some aggregates were still recognized, and a great disruption of surface structure was observed when the product was cooked in boiling

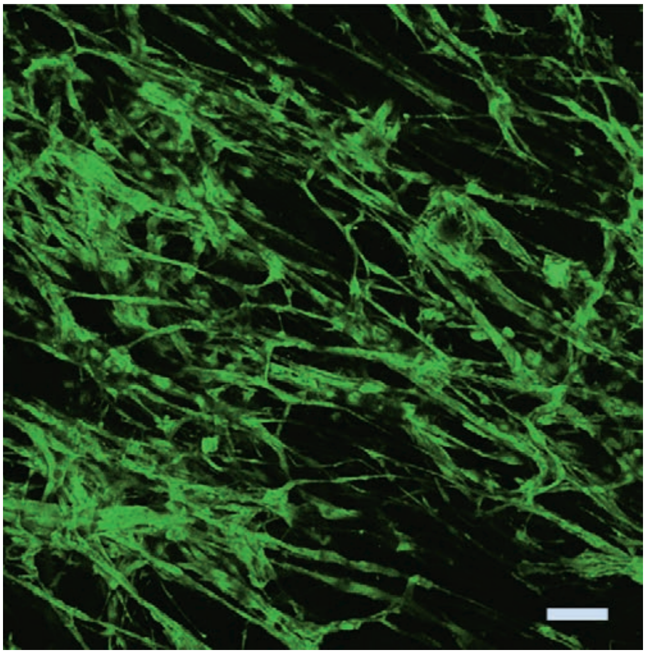

Figure 1. Confocal laser scanning microscopy of gluten network in a slurry sampled at its maximum torque. Sample was stained with Fast Green FCF and observed with excitation /emission wavelengths set at 638 nm/660–740 nm. Scale bar: 20 μm.

water (Marti and Pagani, 2013). Regardless the type of process, the cooking behaviour of 100% rice pasta was very different from durum wheat pasta. Using parboiled rice instead of native or pregelatinized rice was successfully proposed to obtain a product with a more compact internal area (Marti *et al*., 2014b) and enhanced cooking behaviour in terms of texture and cooking loss (Marti *et al*., 2010; Marti *et al*., 2013).

Rice noodles can be considered the "ancestors" of the GF pasta currently available on the market. However, noodles and pasta are produced in different ways resulting in products with different shape, texture, and structure (Bresciani *et al*., 2021). Indeed, melted materials were observed at the surface of rice noodles, while rice starch granules (among the smallest cereals starch granules, mostly 3–5 µm diameter) were still well-recognizable at the surface of rice pasta, indicating a lower influence of the process conditions on starch organization (Lucisano *et al*., 2012).

Even with equal processes, extrusion conditions strongly affect pasta structure. In pasta made of rice and yellow pea (2:1 w/w), the sample processed at low moisture content (28%) presented a rough surface, compared to the smoother surface showed by the samples processed at higher moisture content (30% and 32%) (Bouasla *et al*., 2016). Cross-sectional microstructure revealed an almost homogenous compact inside structure with starch-protein matrix and few aggregates visible at high magnification. On the contrary, samples prepared at low hydration level (28% moisture content) exhibited an irregular inside structure with many aggregates. Insufficient water amount (28%) together with high shearing (100 rpm) during processing led to the formation of irregular inside structures with big empty holes, negatively affecting the overall acceptability of the cooked product (Bouasla *et al*., 2016).

Ultrastructure of GF pasta as affected by formulation

Besides using suitable processing conditions, the choice of appropriate ingredients and/or additives is necessary to obtain a cohesive structure that overcomes the absence of gluten in GF pasta (Marti and Pagani, 2013).

Among cereals, rice is the most frequently used raw material in GF pasta (Morreale *et al*., 2019), because of its abundance, mild taste, high digestibility, and hypoallergenic properties (Rosell and Marco, 2008). The microstructure of GF rice pasta has been mainly characterized using scanning electron microscopy (SEM) and attention has been paid to its surface as well as inner structure because both are generally affected by formulation and processing conditions, including cooking (Bouasla *et al*., 2017; Bouasla and Wójtowicz, 2019; Dib *et al*., 2018). Rice pasta showed a corrugated but smooth surface (Bouasla *et al*., 2017) and the formation of the structure is only demanded to the gelatinization of native flour starch (Lubowa *et al*., 2021). However, rather than being used alone, rice is usually mixed with other ingredients (such as starches, hydrocolloids, emulsifiers and proteins) to enhance the cooking quality of the final product (Marti and Pagani, 2013).

Lubowa *et al.* (2021) investigated the effect of partial substitution of rice flour with pre-gelatinized high-amylose maize starch (combined with sodium alginate) for the production of GF noodles. In this formulation, the structure of the product is the result of a fine balance between gelatinisation of added maize starch and native rice flour starch combined with alginate cross-linking. Specifically, the microstructure of GF noodles was studied observing the inner cross-sectional surface by SEM. The analysis revealed a compact and denser appearance with a lower number of hollows and a continuous matrix in GF noodles enriched with maize starch compared to traditional rice noodles.

As regards proteins, egg albumen and whey proteins were used as texturing ingredient in rice pasta with a positive impact on cooking behaviour (Marti *et al*., 2014). In fact, significantly lower cooking losses were observed in rice pasta with egg albumen or whey proteins compared to GF rice pasta where SEM observations revealed the presence of starch organized in a honeycomb network, also in the central region of cooked pasta (Figure 2a), possibly responsible for its high cooking loss because of the high surface exposure. Differently, starch organized in compact agglomerates trapped between protein fibrils was observed in both protein-enriched samples (Figure 2 b,c).

A continuous network, formed mainly by xanthan and guar gums combined with egg proteins, was observed in quinoa pasta (Sosa *et al.*, 2019). Although not present in the GF pasta available on the Italian market, hydrocolloids or gums can be an easy solution for improving the quality of GF pasta. Indeed, they improve firmness, give body and mouth-feel to GF pasta thanks to their ability to make a gel in little quantity that provide high consistency at room temperature. Moreover, the chemical groups of hydrocolloids are capable to form a stable polymeric network that entraps starch granules, slowing down the amylose release (Padalino *et al.*, 2016).

A decrease in cooking loss is also observed when emulsifiers are included in the formulation (Lai, 2002). Indeed, emulsifiers form a complex with amylose, thus restricting the swelling of starch granules during cooking. The SEM photographs of rice pasta with or without distilled glyceryl monostearate highlighted limited starch gelatinization on the surface of the product (Lai, 2002). This is a desirable feature for GF pasta because it can maintain the shape during cooking and improve its cooking tolerance (Lai, 2002).

Besides rice and corn, other grains - including pseudo-cereals and pulses – are widely used to improve the nutritional profile of the related pasta (Pellegrini and Agostoni, 2015).

The use of pseudo-cereals, such as buckwheat, amaranth and quinoa, as ingredients in pasta formulations, is becoming increasingly popular because they improve the nutritional quality of GF pasta thanks to their high content in fiber, vitamins, minerals and other bioactive components (Alvarez-Jubete *et al.*, 2010).

Microstructure of dry tagliatelle made from quinoa flour has been studied through ESEM by Sosa et al. (2019). Pasta surface was very smooth and homogeneous and the starch granules were part of a continuous network. Quinoa contributed to form a denser protein structure characterized by low porosity that in turn decreased the rate of water diffusion through the matrix. Replacing quinoa flour with corn flour led to some cracks on the surface, that would facilitate water absorption during cooking, indicating that corn seems to interfere the continuous dough formation (Sosa *et al.*, 2019).

Buckwheat-rice mixture (1:1 w/w) allowed the production of GF pasta with a smooth surface and a homogeneous inner matrix with spaces filled with melted starch-protein matrix that keep the structure compact

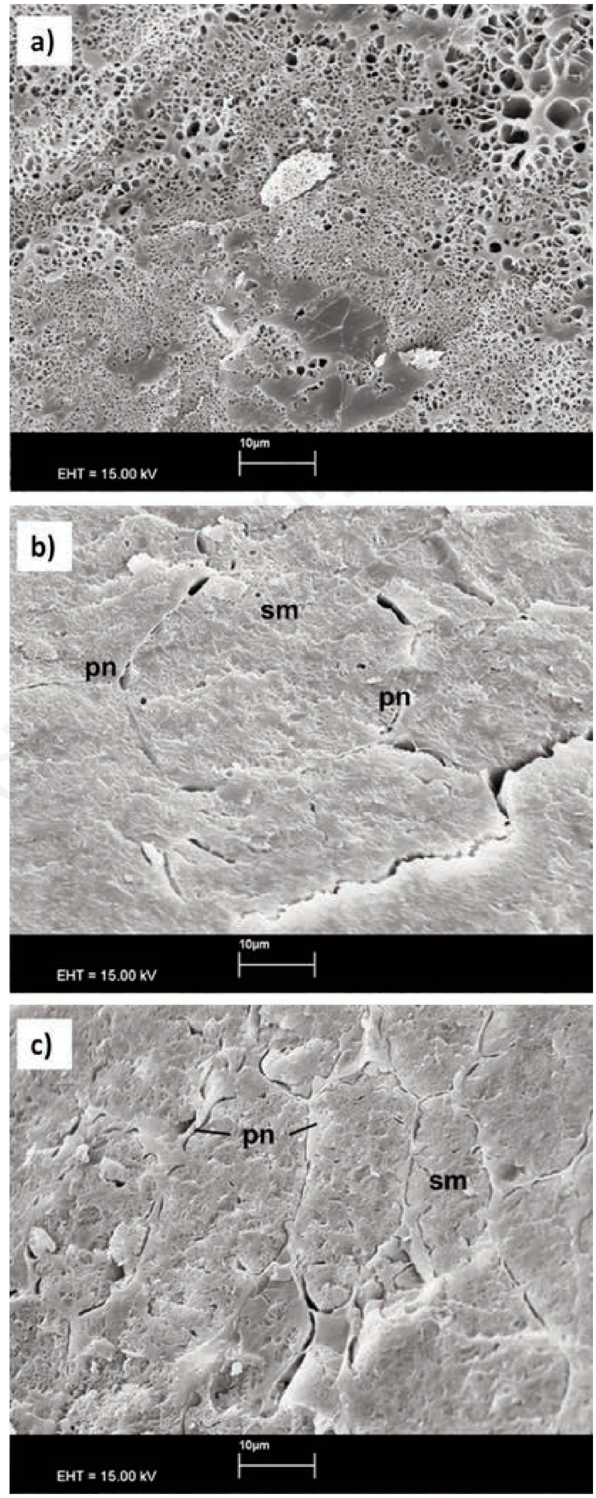

Figure 2. SEM micrographs of cross-sections of cooked pasta at 1000x magnification. Samples of rice pasta (a), rice pasta with egg albumen (b), and rice pasta with whey proteins (c), were sputter-coated with gold after lyophilisation. pn, protein network; sm, starch material.

during cooking (Bouasla and Wójtowicz, 2019). After cooking, the cross-section of the central region was characterized by a compact structure with visible empty spaces resulting from the leaching of components during hot water hydration. Moreover, under high magnification, the external parts of the pasta showed a partially porous structure due to migration of hot water inside pasta. The overall structure supported the low values of cooking loss of the products and the high firmness of the tested pasta (Bouasla and Wójtowicz, 2019).

Legumes (or pulses) represent a good source of proteins, fibers, vitamins, and minerals (Hall *et al*., 2017). Therefore, the incorporation of legume flour in GF pasta would enhance its nutritional quality (Laleg *et al*., 2016). Different legumes have been used alone or in combination with other flours such as rice or corn for GF-pasta production. Common bean, chickpea, faba bean, yellow pea and lentil are among the most used legumes for GF-pasta production (Foschia *et al*., 2017).

Enriching rice pasta with legumes affected pasta surface microstructure by the type of legume (Bouasla *et al*., 2017). In fact, the surface of chickpea-enriched pasta appeared to be corrugated and smooth, likely due to the lubricant effect of high fat content in chickpeas. On the contrary, pasta samples enriched with lentil flour had a rough but not corrugated surface. The cross-sectional microstructure of rice-based pasta did not show differences by the amount and type of legume flours added: a homogenous compact inside structure with starch-protein matrix and few aggregates was observed in all samples. This structure was attributed to the effect of extrusion-cooking conditions, inducing starch gelatinization and the formation of a coherent structure that allowed low cooking loss values (Bouasla *et al*., 2017). After cooking, hydrated rice-chickpea pasta showed a discontinuous surface (Bouasla *et al*., 2017). In particular, the cross-section evidenced empty cavities at pasta surface probably resulting from the leaching of unbounded components to hot water. The number of empty cavities was lower in rice-chickpea pasta compared to rice pasta, likely due to the melted starch-protein matrix formed. As regards beans, Giuberti *et al*. (2015) pointed out the formation of a dense protein and fiber reticules when they were added to rice at both 20 and 40% level. Such structure could have contributed to trap higher amounts of water with respect to control spaghetti, where weaker matrix was identifiable by SEM images.

Finally, Dib *et al*. (2018) used pre-gelatinized rice flour (PGRF) as improver for the production of rice-field bean pasta. Using PGRF determined a more homogeneous distribution of components and a lower amount of visible free starch granules, as evidenced by SEM observations of pasta cross-sections. PGRF was able to form aggregates combining all pasta components, and the continuous matrix of gelatinized rice starch was responsible for the limited leaching of components during cooking and the enhanced consistency of GF pasta enriched with bean.

Conclusion

This work showed that the study of ultrastructure in GF pasta is a helpful method of investigation to obtain a final product with good quality and cooking performances. It provides key information in identifying the best formulation and optimizing manufacture conditions. Microscopy approach, SEM in particular, plays an important role in understanding how other ingredients than gluten affect final product texture and the interaction among food components at the different stages of pasta making process.

The diverse combinations of ingredients used in GF-pasta production may offer the consumers pasta with different technological and nutritional quality. In this respect, understanding the relationship between pasta structure and formulation and/or processing conditions might help in "designing" new food products.

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