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M. A. Pagani

D. J. Gallant

B. Bouchet

P. Resmini

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ULTRASTRUCTURE OF COOKED SPAGHETTI

M.A. Pagani, D.J. Gallant*, B. Bouchet*, P. Resmini

ISTITUTO DI INDUSTRIE AGRARIE, UNIVERSITA DEGLI STUDI
20133 Milano, Italy

*INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE
Laboratoire de Technologie des Aliments des animaux
Unité Microscopie
rue de la Géraudière - 44072 Nantes Cedex, France

Abstract

Several electron microscopy (EM) complementary techniques (scanning electron microscopy, freeze-fracturing and thin-sectioning) have been applied in the ultrastructural study of spaghetti.

Experimental spaghetti have been produced starting from two semolinas from the same wheat cultivar and using a low temperature (LT) and very high temperature (VHT) drying schemes.

Cooking quality of these products was not related to the quantity of the main components present in semolina. However, the drying conditions (temperature and humidity) and the nature of the cooking water greatly influenced cooking characteristics.

The three EM techniques were used to detect differences in protein and starch organization in relationship to spaghetti quality. Structural differences present in the uncooked product were more evident after cooking. In particular, in each high quality spaghetti, interesting macromolecular arrangements were always found inside starch granules. These new structures, which were dramatically promoted by VHT drying, exhibited an exceptional resistance to alpha-amylase digestion.

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Direct inquiries to D.J. Gallant
Telephone number: 33 40 76 23 64

Key words : Spaghetti, wheat flour, drying conditions, pasta cooking quality, scanning electron microscopy, transmission electron microscopy, freeze-fracturing, thin-sectioning, cytochemistry.

Introduction

The characteristics and texture of cooked pasta are strongly affected by semolina protein quality and quantity (D'Egidio et al., 1979; Frey and Holliger, 1972; Grzybowski and Donnely, 1979; Matsuo et al., 1972; Wasik and Bushuk, 1975), drying conditions (Dexter et al., 1981 a and b; Manser, 1979; Resmini and Pagani, 1983; Wyland and D'Appolonia, 1982), and by the nature of the cooking water (Alary et al., 1979; D'Egidio et al., 1981; Dexter et al., 1983).

Starch gelatinization and swelling and protein coagulation occur during pasta cooking (Resmini and Pagani, 1983). These phenomena occur at approximatively the same conditions of temperature and moisture and are competitive and antagonist (Frey and Holliger, 1972; Resmini and Pagani, 1983). At present we do not have a complete understanding of the biochemical and biophysical factors affecting protein coagulation or starch swelling.

Electron microscopy (EM) techniques allow one to observe the organization of pasta components. Thus, they represent good tools to obtain informations about protein and starch changes which are promoted by different pasta-making technologies. Also, EM helps to evaluate interactions that occur between the two components during pasta cooking.

Materials and Methods

Semolina composition

Protein content was determined by the Kjeldhal method (N X 5.7). Starch content was evaluated according to Thivend et al. (1972); amylose determination was carried out by amperometric titration (Larson et al., 1953). Ethanol-soluble carbohydrates (80% aqueous ethanol) were measured as proposed by Mercier and Feillet (1975); total lipid content according to Drapron (1975).

Spaghetti production

All spaghetti samples were produced at the "Laboratoire de Technologie des Céréales", I.N.R.A., in Montpellier (France), starting from French semolina of Mondur cultivar grown in two different places (Montpellier and Niverville).

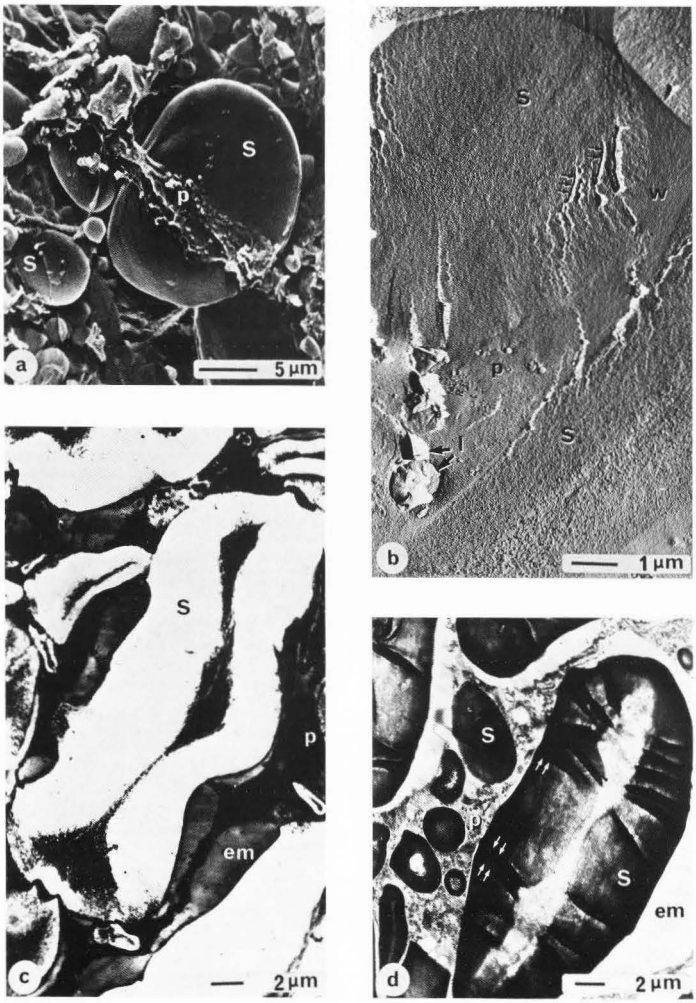


Fig. 1. Commercial uncooked spaghetti. a) SEM image; b) FF image; c) thin-section (uranyl acetate/lead citrate staining); d) thin-section (PATAg staining). Small arrows indicate artifacts due to the fracture (Fig. 1b) and to folds (Fig. 1d). (S) starch granule; (p) protein matrix; (l) lipid inclusions; (w) water; (em) embedding medium.

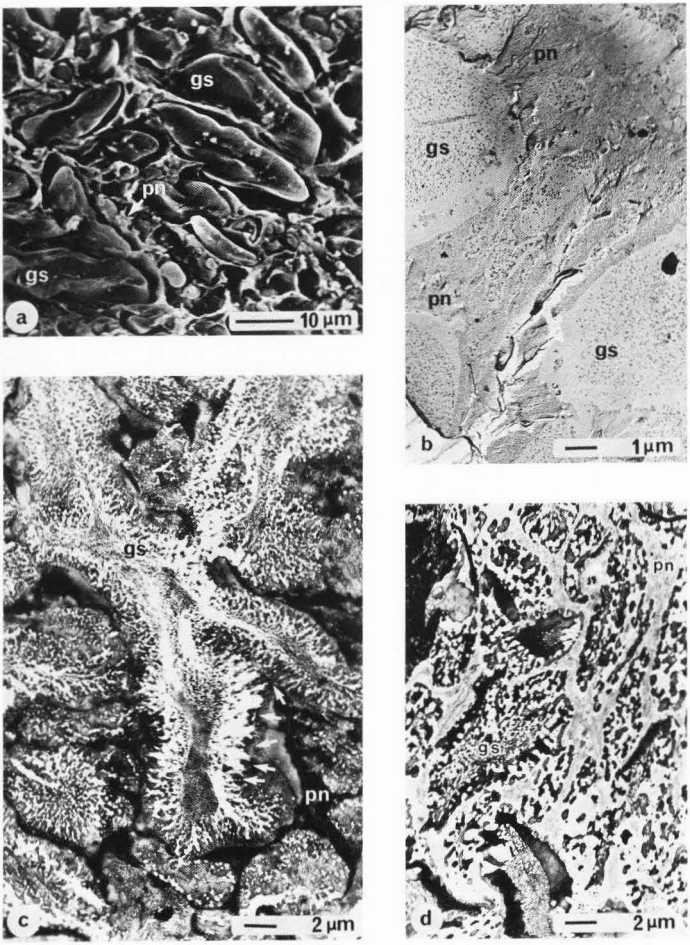


Fig. 2. Commercial cooked spaghetti. a) SEM image; b) FF image; c) thin-section (uranyl acetate/lead citrate staining); d) thin-section (PATAg staining). (gs) gelatinized starch; (pn) protein network; white arrows (Fig. 2c) indicate alveoles.

Spaghetti was prepared in a Demaco scale laboratory press and dried by a low temperature (LT) drying method in a laboratory scale drying cell equiped with an automatic system for ventilation and resting. Spaghetti Mondur Niverville was also dried at very high temperature (VHT), a few hours over 100°C according to an unknown diagram because of a damage to the drier control system. This spaghetti resulted in changes in colour and taste but showed so interesting cooking properties that its ultrastructural studies seemed quite advisable.

Spaghetti cooking test

5 g of spaghetti (strands of 2 cm) were plunged into 150 ml of boiling mineral water (Evian water, pH 7.2) (Alary et al., 1979) and stirred for the first 3 minutes. After 5 minutes of cooking, few strands were taken out and drained; the rest of the spaghetti was cooked to the optimal cooking time (Dexter et al., 1981b). Cooking quality was evaluated as proposed by Alary et al. (1979) taking into consideration stickiness, aspect and viscoelasticity properties. LT Mondur Niverville spaghetti was also cooked in boiling distilled water (pH 5.5) (Alary et al., 1979) to assess the cooking quality improvement observed by several authors (Alary et al., 1979; D'Egidio et al., 1981; Dexter et al., 1983).

Ultrastructure studies

All the uncooked spaghetti was soaked for 12 hours in aqueous media prior to each EM preparation. Soaking was carried out in 30% glycerol-water for freeze-fracturing (FF) and in mineral water for thin-sectioning and SEM.

SEM specimen preparation. Uncooked and cooked spaghetti were examined both at the surface and in transversal section. Samples were fixed one hour in 6% and one hour in 3% glutaraldehyde solutions in 0.1M Na cacodylate buffer (pH 7), dehydrated in graded acetone series, critical point dried, mounted on stubs and shadowed with gold (Ion Sputtering JEOL JFC 1100; thickness of gold layer : 40 nm). Samples were observed in a JEOL 50A scanning electron microscope at an acceleration voltage of 20 keV.

TEM specimen preparation.

Thin-sectioning. Sections of uncooked and cooked spaghetti were fixed differently according to the staining technique. For carbohydrate staining, samples were fixed one hour in 6% and one hour in 3% glutaraldehyde solutions in 0.1M Na cacodylate buffer (pH 7). For protein staining, after the fixation in glutaraldehyde solutions as described above, the specimen were post-fixed one hour in 1% buffered OsO_4 solution in order to obtain better contrast for protein and lipid.

The fixed specimens were dehydrated in graded acetone solutions, embedded in Epon and sectioned using a diamond knife in a JEOL JUM 7 ultramicrotome (thickness of specimen 70-100nm). The thin-sections were placed either on copper or gold grids supported with a collodion membrane and stained. The staining with uranyl acetate and lead citrate solutions (Bechtel et al., 1978; Frey and Holliger, 1972) is selective for protein and lipid which appear dark grey or black. With PATAg staining technique (Gallant, 1974; Gallant and Guilbot, 1969) starch and other carbohydrates appear black. The stained thin-sections were observed in a JEOL 100S transmission electron microscope at 80 or 100 keV.

Freeze-fracturing. Replicas were prepared in the conditions recently proposed by Resmini and Pagani (1983). Spaghetti samples were frozen in super-cooled liquid nitrogen, transferred into a BALZERS FF unit (BAF 301) heated to -95°C, fractured at -105°C and shadowed with Pt/C film immediately after the fracture. Replicas, after suitable cleaning in H_2SO_4 solutions, distilled water, acetone and bidistilled water, were observed in a JEOL 100S transmission electron microscope at 60 or 80 keV.

Enzymatic studies

To understand the nature of the particular structures, the thin-sections were treated with enzymes and stained as described above to identify proteins and carbohydrates.

Alpha-amylase hydrolysis. The grid with the section was exposed to a 0.1% buffered amylase solution (alpha-amylase from Bacillus subtilis, Boehringer; 0.1M phosphate buffer, pH 7.2). The amylolysis was carried out for 15 minutes at room temperature.

Proteolitic hydrolysis. The grid with the section was exposed to a 0.2% buffered pronase solution (pronase from Streptomyces griseus, Boehringer; 0.1M phosphate buffer, pH 7.5). The proteolysis was carried out for 30 minutes at 40°C.

Results and Discussion

Advantages and disadvantages of the different techniques used in EM

Each EM technique offers advantages and disadvantages in the ultrastructural study of spaghetti. Preparations are generally simple in scanning electron microscopy (SEM). Before cooking, starch granules (size ranging from 2 to 40 µm) can be clearly seen below the protein matrix (Fig. 1a). The protein, because of hydration (Chabot, 1979; Dexter and Matsuo, 1977; Dexter et al., 1978), appears organized in a fibrillar network (the gluten) which surrounds the starch granules. But SEM shows only surface morphology (Angold, 1979; Davis and Gordon, 1983; Hsieh et al., 1981) and the three dimensional organization. Furthermore SEM preparation methods produce artifacts due to chemical fixation and dehydration (Chabot, 1979; Davis and Gordon, 1983; Varriano-Marston, 1981).

Transmission electron microscopy (TEM), methods such as freeze-fracturing (FF) and thin-sectioning techniques, allow the investigation of internal fine structures. In a traditional spaghetti before cooking, we can observe by FF (Fig. 1b) the compact spherulitic structure of the starch granules, the protein matrix organized into subunits, and the uniformly dispersed protein among the starch granules and containing numerous lipid inclusions. The information given by this technique is of extreme interest. FF is recognized as one of the most suitable techniques in food ultrastructure investigations since it does not introduce artifacts due to chemical fixation and/or dehydration (Chabot, 1979); Davis and Gordon, 1983; Fretzdorff et al., 1982a; Resmini and Pagani, 1983). On the other hand, information and interpretation of pasta FF images require considerable experience. The identification of starch and proteins is made easily after

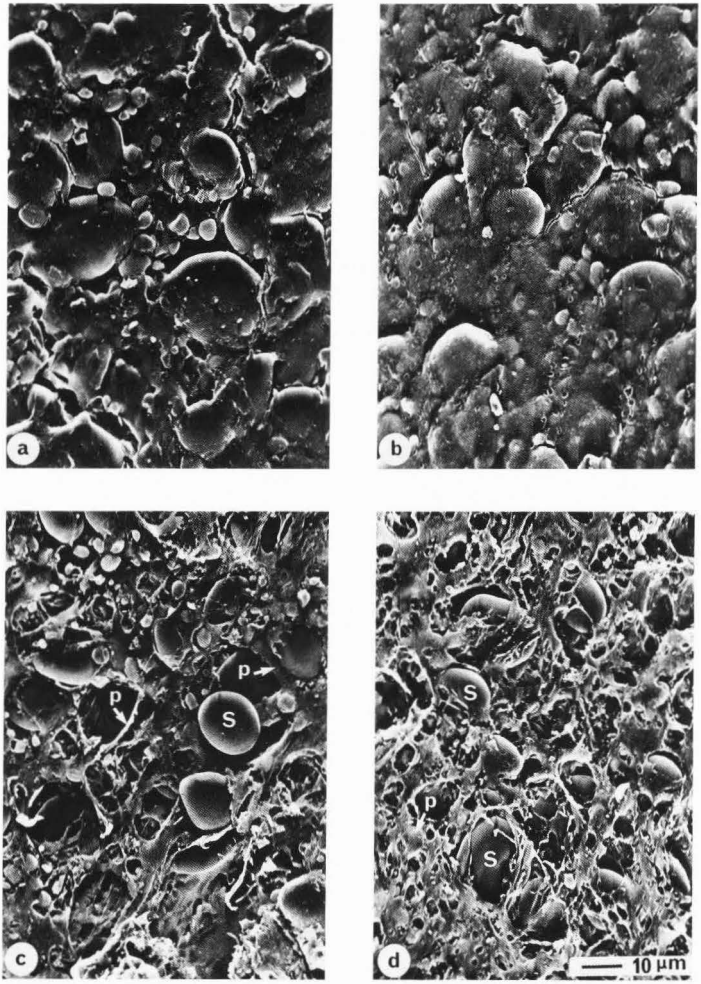
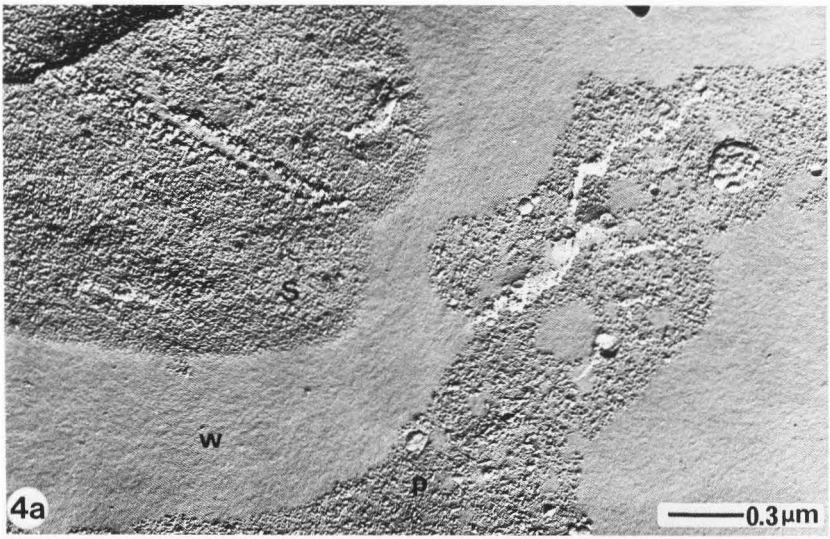


Fig. 3. SEM images of experimental uncooked spaghetti. a), b) before hydration; c), d) after hydration. a), c) MM, good quality spaghetti; b), d) MN, poor quality spaghetti. (p) protein matrix; (S) starch granule.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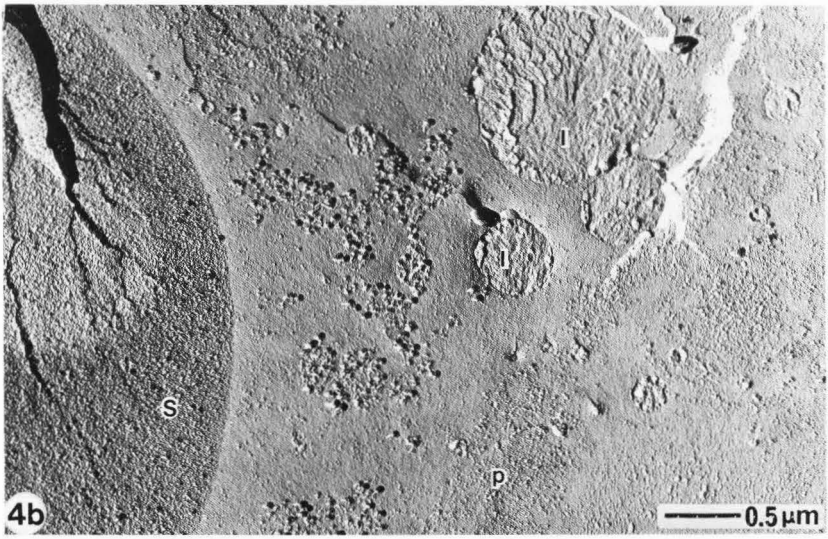
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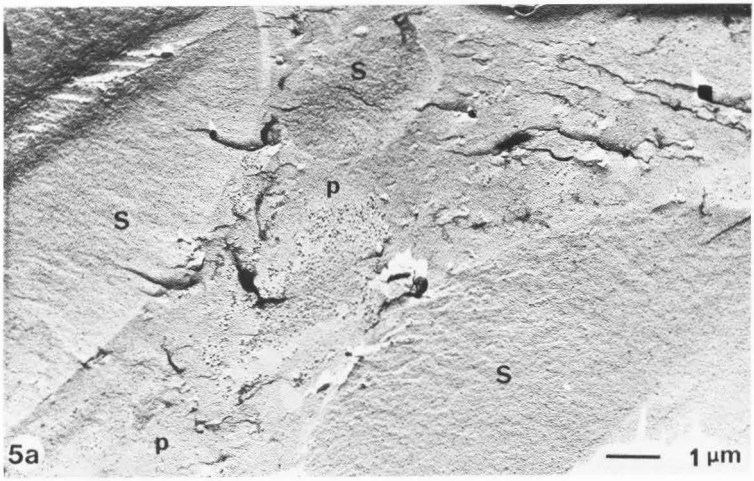
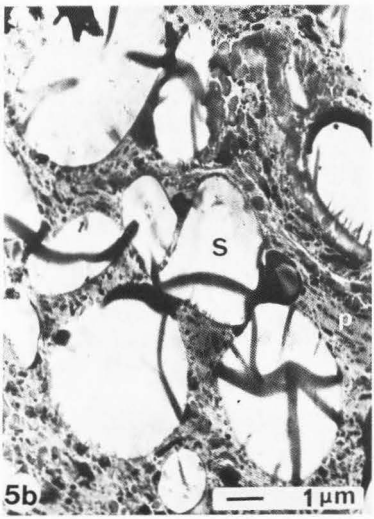




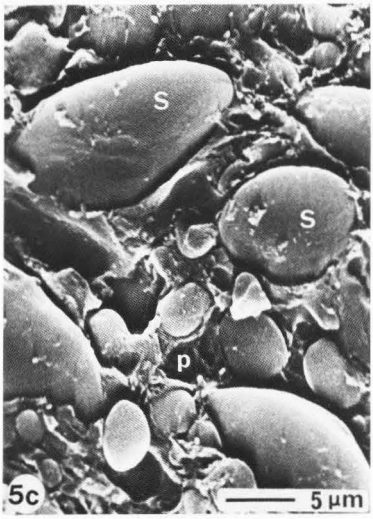


Fig. 4. FF images of experimental uncooked spaghetti. a) MM, good quality spaghetti; b) MN, poor quality spaghetti. (l) lipid inclusions; (p) protein matrix; (S) starch granule; (w) water.

Fig. 5. Uncooked MN-VHT, high quality spaghetti. a) FF image; b) thin-section (uranyl acetate/lead citrate staining); c) SEM image. (p) protein matrix; (S) starch granule.

a careful study of the isolated components, before and after cooking (Fretzdorff et al., 1982a; Resmini and Pagani, 1983).

TEM which involves thin-sectioning and specific staining for proteins and starch, provides immediate identification of these components. The staining with uranyl acetate/lead citrate is selective for proteins and lipids and appears as markedly grey (Fig. 1c) (Gallant, 1974; Bechtel et al., 1978; Fretzdorff et al., 1982 b; Hood and Liboff, 1983); the staining with silver salts (PATAg technique, Fig. 1d) identifies starch granules and all other carbohydrate components, giving them the black, characteristic, punctuated image (Gallant, 1974; Gallant and Guilbot, 1969; Duprat et al., 1980; Gallant and Sterling, 1976; Fretzdorff et al.,1982b). However thin-sectioning methods may cause artifacts due to the indispensable chemical fixation and dehydration steps as with SEM. It is impossible to determine the actual water distribution in thin-sections and by SEM (Fretzdorff et al., 1982a). Another disadvantage of thin-sectioning, also common to FF techniques, is the difficult and the tedious specimen preparation, especially for cereal products (Hsieh et al., 1981).

Same comments may be extended to the spaghetti ultrastructures observed after cooking by the different techniques. Morphologic changes of starch granules as a consequence of their water absorption and swelling can be observed by SEM (Fig. 2a). But it is important to keep in mind the artifacts introduced by the specimen preparation (e.g., in our case dehydration by critical point drying). The more the granules are swollen, the greater will be their deformation. In fact dehydration always produces granule shrinkage (Chabot et al., 1979; Christianson et al., 1982; Hood and Liboff, 1983). A network consisting of coagulated proteins surrounds the swollen starch granules.

TEM provides informations on fine component modifications. Thin-sectioning shows (Fig. 2c, 2d) the developing of alveoles containing solubilized material inside the swelling granules. However, it is impossible to follow protein changes. Using TEM in fact, protein appear as a smooth compact matrix, both in uncooked and cooked products. Conversely, the examination of FF ultrastructure of cooked spaghetti reveals interesting details (Fig. 2b). Starch swelling and gelatinization show the native spherulites. The native protein subunits interact and coagulate into a network which, if continuous, may retain and contain the swelling granules (Resmini and Pagani, 1983). Pasta drying and/or cooking produce changes in the three-dimensional arrangement and in the internal organization of proteins and starch. Integration of the microstructural methods (SEM, FF and thin-sectioning) provides important information to scientists engaged in this field of study. Furthermore, the possibility of comparing the microstructure of the same sample using several techniques makes it possible to detect artifacts in order to avoid misinterpretations.

Table 1. Cooking characteristics of experimental spaghetti.

SEMOLINA	DRYING	COOKING LIQUID	OPTIMAL COOKING TIME (min)	COOKING QUALITY
MONDUR Montpellier	LT	Mineral	10	Good
MONDUR	LT	Mineral	10	Poor
Niverville	LT	Distilled	10	Good
	VHT	Mineral	18	High

Therefore, the purpose of this work was only to investigate the ultrastructure of experimental spaghetti of different cooking quality by FF, thin-sectioning and SEM techniques and to relate some spaghetti microstructured observations with cooking behaviour.

Characteristics of experimental spaghetti

As shown in table 1, the experimental spaghetti shows different cooking qualities. Even if the quantity of the main components present in semolina is recognized as an important factor in determining pasta cooking characteristics (Grzybowski and Donnely, 1979; Matsuo and Irvine, 1970; Matsuo et al., 1972), the differences in quality tested on our samples after cooking are not related to the quantitative composition. Protein, starch and lipids contents were quite similar (Table 2) in the two semolinas.

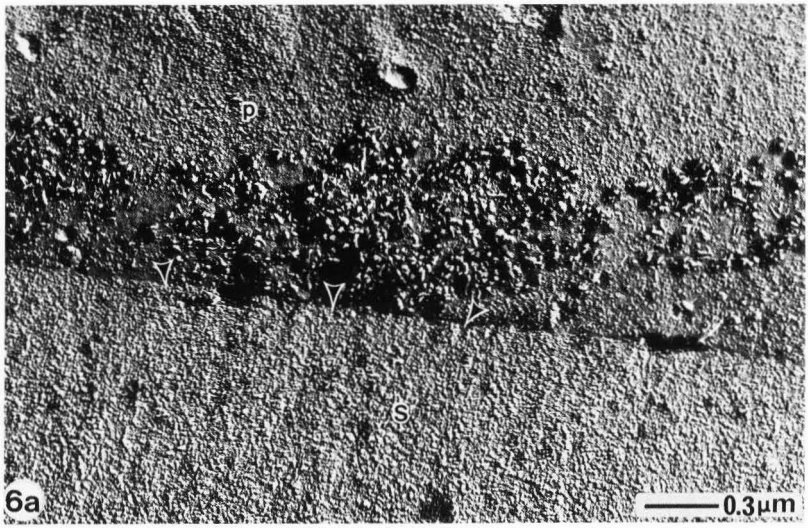
Good cooking properties as determined by a sensory panel may provide by the use of good semolina (Mondur Montpellier-low temperature = MM) but, if not the case (Mondur Niverville-low temperature = MN), to improve it by inducing water acidity (MN-distilled water = MN-dw) or the use of very high temperature (MN-VHT) during spaghetti drying schemes.

Ultrastructure of experimental spaghetti before cooking

When we examined surface of dry spaghetti products by SEM, one observed the starch granules embedded into protein matrix (MM, Fig. 3a), the poor quality showing more pasty aspect (MN, fig. 3b). As stated by Angold (1979) starch and protein components are difficult to identify, a continuous film, probably of proteic nature enveloping and hidding the starch granules (Dexter et al., 1978).

Hydration of uncooked samples may introduce artifacts, especially in protein organizating, but this preparation step allows a better correlation with FF images (Chabot, 1979) and provides a clearer identification of spaghetti components (Resmini and Pagani, 1983). Hydration of uncooked spaghetti did not cause changes in starch ultrastructure (Matsuo et al., 1978; Resmini and Pagani, 1983) but promoted a compact protein structure in the good quality spaghetti (Fig. 3c) and porous protein structure in the poor quality one (Fig. 3d). A protein matrix showing the characteristics of the latter sample may certainly promote a quicker water absorption at the beginning of cooking.

Fig. 6. FF ultrastructure of uncooked MN poor quality spaghetti. a) after VHT drying; b) after LT drying. Arrows indicate probable starch-protein interactions (Fig. 6a) and water coat (Fig. 6b). (l) lipid inclusions; (p) protein matrix; (S) starch granule.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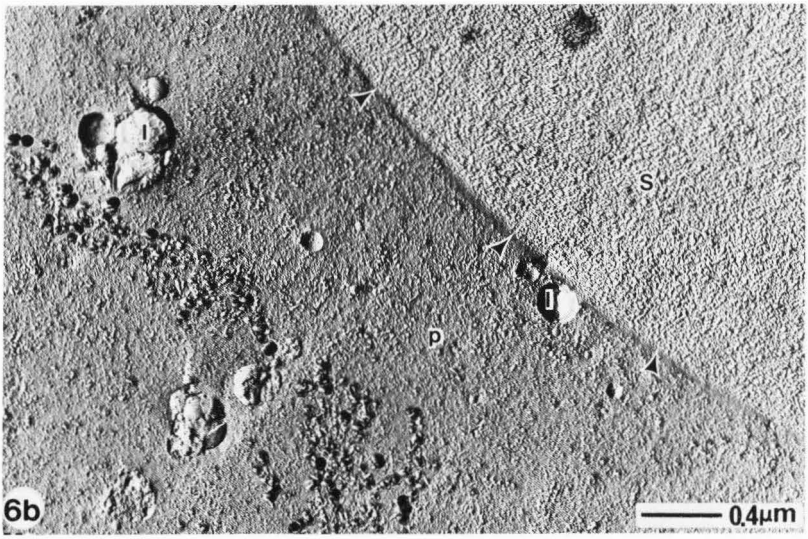
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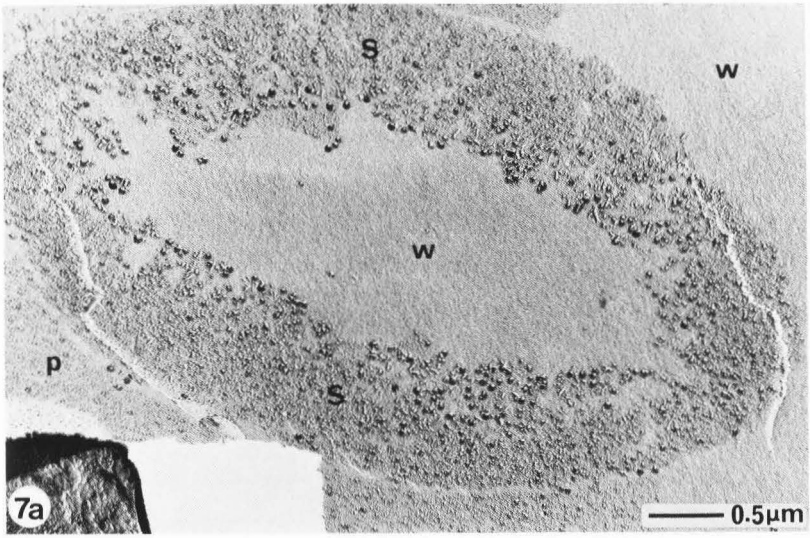

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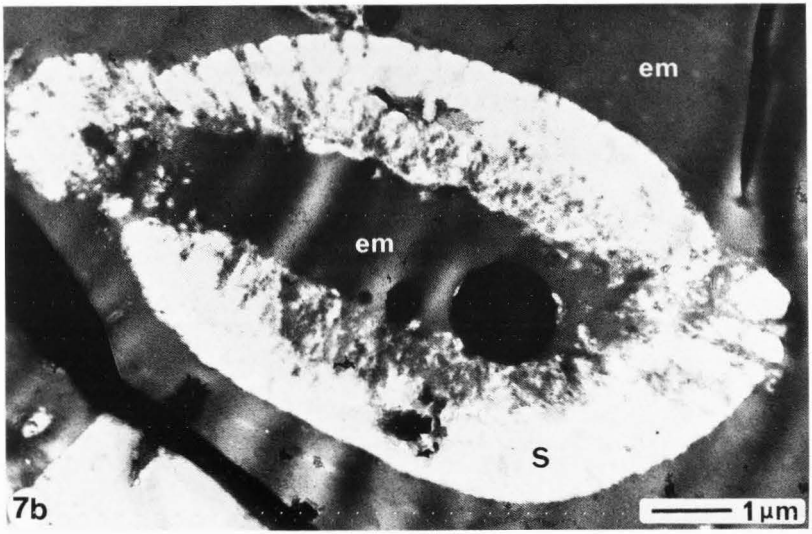

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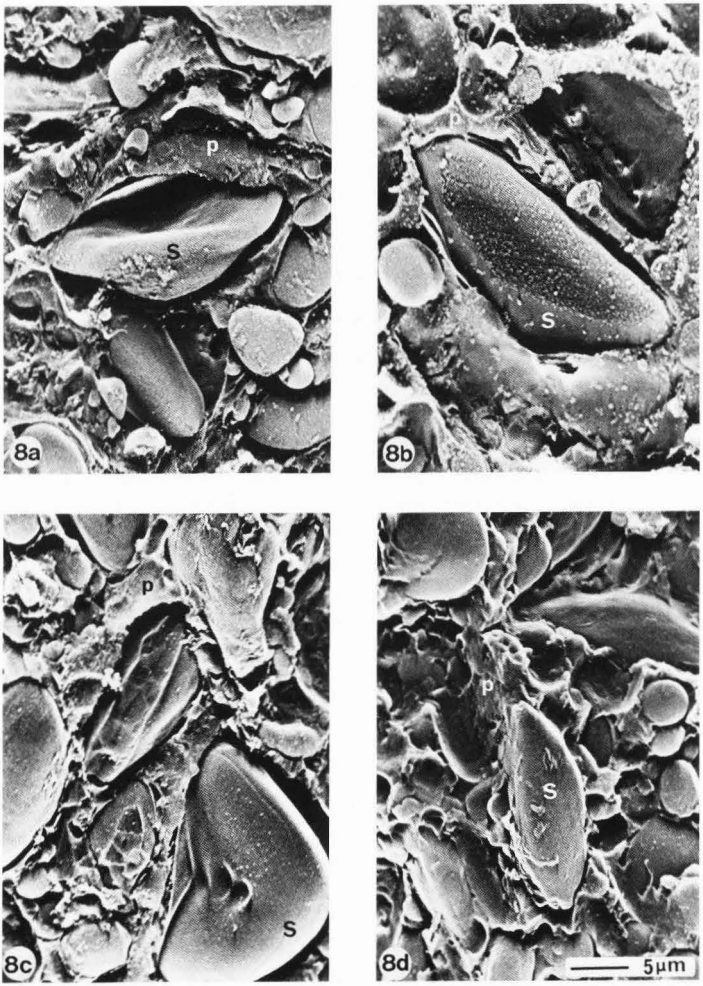

Fig. 7. Starch damage in MN, poor quality spaghetti. a) FF image; b) thin-section (uranyl acetate/lead citrate staining). (em) embedding medium; (p) protein matrix; (S) starch granule; (w) water.

Fig. 8 SEM images of experimental spaghetti after partial cooking. a) MN, poor quality spaghetti; b) MM, good quality spaghetti; c) MN-dw, good quality spaghetti; d) MN-VHT, high quality spaghetti. (p) protein matrix; (S) starch granule.

Table 2. Semolina composition (g/100 g d.m.)

SEMOLINA	PROTEIN (N x 5.7)	STARCH	AMYLOSE (*)	ETHANOL-SOLUBLE CARBOHYDRATES	LIPID
MONDUR Montpellier	14.8	75.5	28.2	1.8	2.0
MONDUR Niverville	15.2	74.1	27.7	1.8	2.3

(*) g/100 g of starch.

The same differences in the protein pattern of good (MM) and poor (MN) spaghetti quality were observed in FF micrographs (Fig. 4). These images were less impressive than SEM ones but we could detect as well the interactions among protein subunits which may have formed during semolina kneading. These interactions resulted much more extensively in the good quality product (MM, Fig. 4a). We could correlate these protein patterns with the SEM protein structures observed by Matsuo et al. (1978) in doughs of the same semolina kneaded with different water contents. Based on the images presented by these authors, we can assume that in poor sample (MN) the quantity of water normally used in kneading (about 30%) was insufficient for a complete gluten development, as opposed to what occurred in good quality products.

The exceptional cooking properties of MN-VHT spaghetti (Table 1) are related to a particular protein features (Fig. 5). As stated in the recent study of Resmini and Pagani (1983), high temperature drying promotes the coagulation of protein fractions into a continuous network, clearly visible in FF (Fig. 5a), TEM (Fig. 5b) and SEM (Fig. 5c) micrographs, and that prevents excessive starch swelling and subunits scattering during cooking. All three EM techniques pointed out the substantial compactness of MN-VHT spaghetti, indicating that a slower water penetration and a longer optimal cooking time (Table 1) are required. In FF micrographs (figs. 5a and 6a) proteins took up the area where a water coat (at least 30 nm wide) generally surrounded the starch granules in a spaghetti dried at low temperature (Fig. 6b). These structures may represent the starch-protein interactions observed by Resmini and Pagani (1983) in spaghetti cooked after an oven treatment. The effects of this VHT diagram on cooking quality and ultrastructure and by Resmini's oven treatment were quite similar.

Cooking quality is also affected by starch behaviour (D'Egidio et al., 1983; Dexter and Matsuo, 1979; Resmini and Pagani, 1983). Enzymatic and/or mechanical damage to starch granules during pasta making induce negative cooking characteristics (Lintas and D'Appolonia, 1973; Matsuo et al., 1982). Therefore, the poor quality of MN spaghetti may be also related to damaged starch granules as suggested in FF (Fig. 7a) and thin-sectioning (Fig. 7b) micrographs when appear to have a solubilized inner fraction, the center part representing the most susceptible area for enzymatic digestion (Chabot, 1979; Gallant, 1974; Gallant and Guilbot, 1969).

Ultrastruture of experimental spaghetti after partial cooking

Because of the physical competition between starch gelatinization and protein coagulation during cooking (Resmini and Pagani, 1983), shorter cooking times may give important information about the kinetics of these phenomena and may explain the cooking results. As shown by SEM in Fig. 8a, in the inner part of poor quality spaghetti (MN) after only 5 minutes of cooking, the larger starch granules, which are believed to gelatinize at a lower temperature, already swollen while the protein matrix is not yet completely coagulated.

In all the good (MM, Fig. 8b; MN-dw, Fig. 8c) and high (MN-VHT, Fig. 8d) quality spaghetti, coagulated protein, promoted by native protein characteristics (Fig. 8b) or by high temperature treatment (Fig. 8d) or induced by water acidity (Fig. 8c), prevailed over gelatinized starch.

Ultrastructure of experimental spaghetti after optimal cooking

Cooking to the optimal cooking time clearly shows that there is competition for water to gelatinize starch and coagulate protein. Stickiness and absence of firmness exhibited by poor quality MN spaghetti (Fig. 9) are the consequences of starch swelling preferentially and not being prevented during continuous protein network formation as seen by complementary techniques (Fig. 9) particularly spherulites scattering seen by FF (Fig. 9b), and alveoles rising as seen by TEM (Fig. 9c and 9d). Furthermore, the complete alpha-amylolysis (Fig. 9e) of the starch material contained in the alveoles and formed during cooking, suggested that starch was completely gelatinized and not at all retrograded (Hansen and Jones, 1977; Shetty et al., 1974). Grey alveoles-like which were seen in the swollen starch, completely hydrolyzed by alpha-amylolysis treatment of ultrathin-sections correspond to the embedding material that had penetrated the swollen starch granules before polymerization.

Cooking good and high quality spaghetti (MM, MN-dw and MN-VHT; Fig. 10) did not cause the spherulites scattering (Fig. 10d) and the alveoles rising (Fig. 10a, 10b and 10c), because of the presence of a thin, continuous protein network, which is an essential characteristic of a good product. All the TEM micrographs (Fig. 10, 11a and 11b) showed specific fibrillar and ordered structures in every starch granule. Similar arrangements have been recently observed by Resmini and Pagani (1983) in replicas of excellent cooking quality spaghetti and by Fretzdorff et al. (1982a) in replicas of bread crumb. In

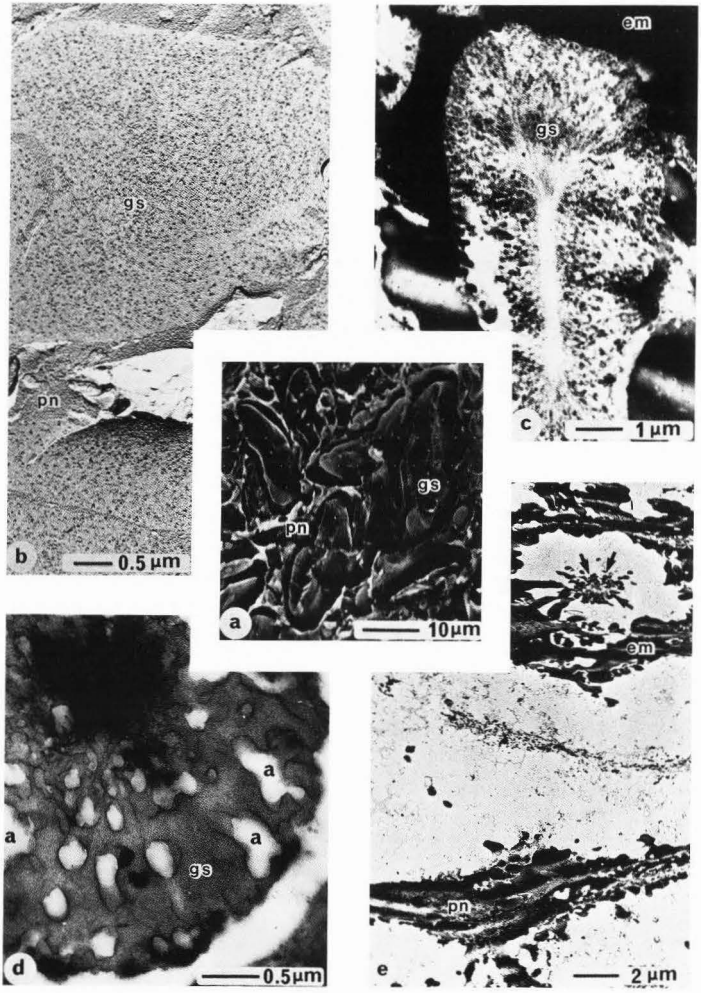

Fig. 9. MN, poor quality spaghetti cooked in mineral water to the optimal cooking time. a) SEM image; b) FF image; c) thin-section (uranyl acetate/lead citrate staining); d), e) thin-sections (PATAg staining). At high magnification, aleveoles (a) are seen in the swollen starch granule (Fig. 9d). When gelatinized starch is completely hydrolysed after alpha-amylolysis of the thin-section (Fig. 9e) it only remain grey alveoles-like structure (black arrows) which correspond to the embedding material that had penetrated the swollen granules. (em) embedding medium; (gs) gelatinized starch granule; (pn) protein network.

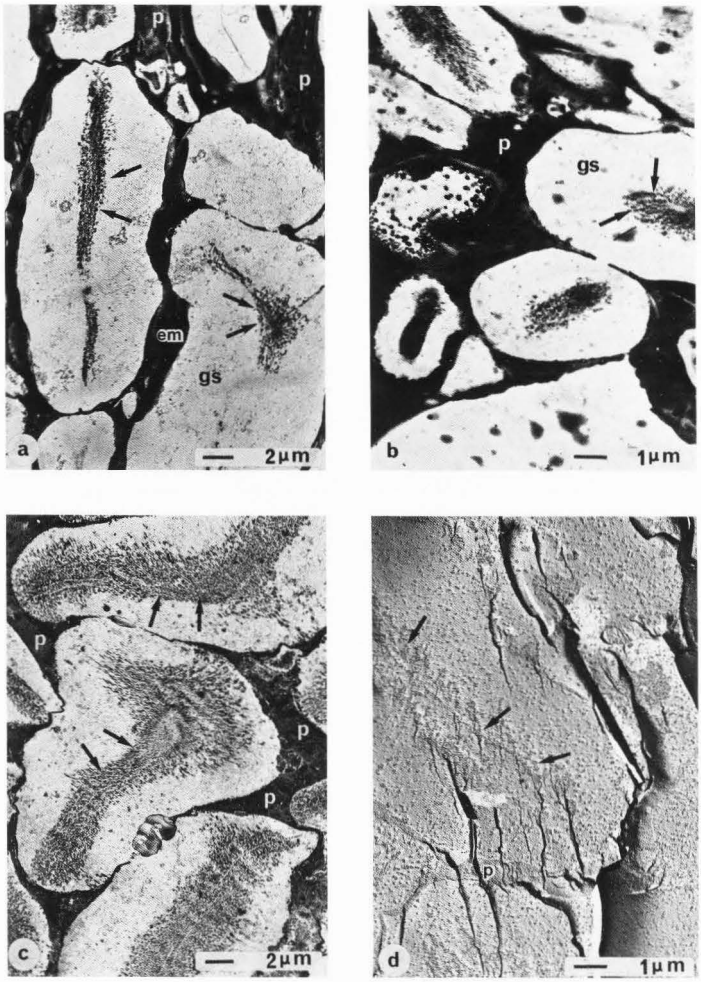

Fig. 10. TEM micrographs of experimental spaghetti cooked to the optimal cooking time. a) thin-section of MM good quality spaghetti; b) thin-section of MN-dw, good quality spaghetti; c) thin-section of MN-VHT, high quality spaghetti; d) FF image of MN-VHT, high quality spaghetti. In this kind of product we observe no alveoles rising (see Fig. 2c). On the contrary particular groupings and macromolecular arrangements are present inside each starch granule (arrows). All the thin-sections are stained with uranyl acetate/lead citrate. (em) embedding medium; (gs) gelatinized starch granules; (p) protein.

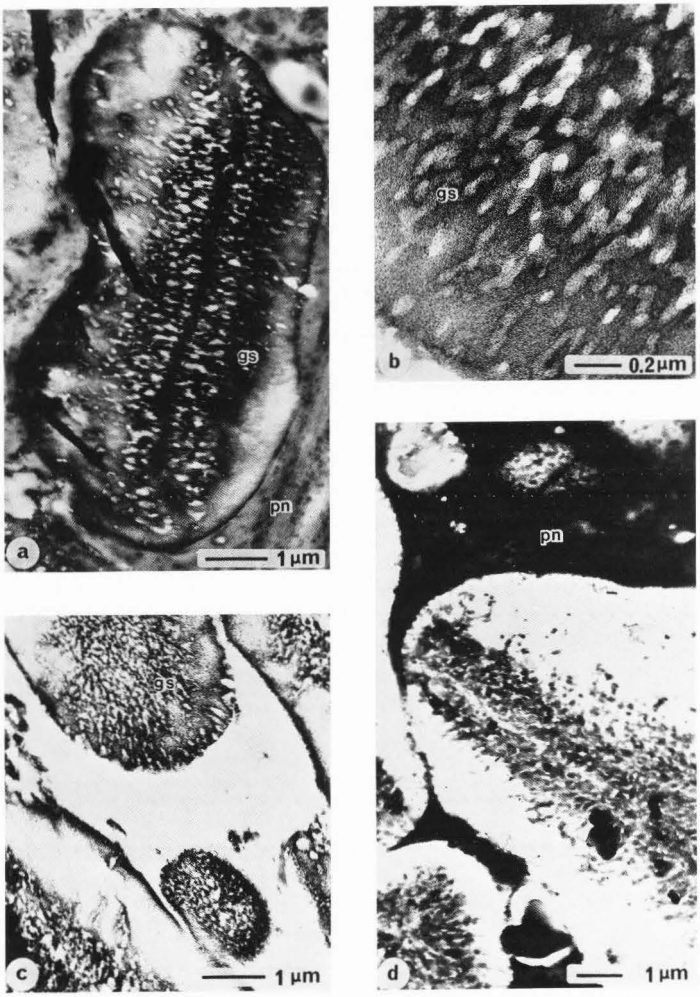

Fig. 11 Thin-sections of cooked MN-VHT, high quality spaghetti after PATAg staining. a), b) show the starchy nature of the macromolecular arrangements. These groupings, despite contrasted as proteins in Fig. 10, are kept intact by pronase (c) and present a strong resistance to alpha-amylolysis (d). (gs) gelatinized starch granules; (pn) protein network.

thin-sections the nature of starch in these samples was dubious because staining revealed protein structure of similar contrast (Fig. 10a, 10b and 10c). Enzymatic hydrolysis using pronase (Fig. 11c) or alpha-amylase (Fig. 11d) clarified the origin of the macromolecular forms. Pronase caused a complete hydrolysis of the protein network (Fig. 11c, white area) leaving the starch granules intact. The starch exhibited an exceptional resistance to alpha-amylase digestion (Fig. 11d), typical of retrograded starch (Banks and Greenwood, 1975; Collison, 1968). Therefore, in all the good and high quality samples, starch had undergone some retrogradation, particularly after ultra high temperature treatment (Fig. 10d). Donovan et al. (1983) also observed new physico-chemical properties in starch after heat--moisture treatments and suggested the formation of new crystalline structures, eventhough they undergo no processing treatments.

Conclusion

Pasta quality can not be ascertained by determining the chemical composition or the ultrastructure of the uncooked product. Nevertheless the spaghetti ultrastructure before cooking is useful in determining changes occurring as a result of processing that would have a positive or negative effect on cooking quality (e.g. protein coagulation during high temperature drying, or starch damage during kneading and drying). Freeze-fracture techniques are useful in such studies. Thin-sectioning methods, even if interesting, may produce artifacts due to chemical fixation.

After cooking, pasta quality is characterized by two different ultrastructural models. In a sticky product, starch gelatinization overcomes protein reticulation. In contrast, in a firm spaghetti, proteins coagulate forming a continuous network around each starch granule. The SEM images allow one to observe growth of component changes. But the fine modifications of protein and starch during cooking, which are strictly related to pasta quality, can be appreciated only by a TEM technique, for example in studying the macromolecular aggregation inside the starch granules in good spaghetti detected by FF.

EM observations supply ideas of factors affecting pasta quality. However, in order to truly understand the relationship to component characteristics, one must also relate them to chemical, physico-chemical and physical evaluations.

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Discussion with Reviewers

E.A. Davis: Were the starch granules sensitive to electron beam damage for SEM samples, when using an accelerating voltage of 20 keV ?
Authors: When accelerating voltage of 20 keV is used with very low current (as 10^{-12} to 10^{-13} ampere) starch granules are not sensitive to electron beam damage.

R.R. Matsuo: Since the chemical composition of the two samples is essentially the same, differences in cooking quality must be attribuable to environmental effects. That is to say, the sample from Niverville must have been damaged, e.g. weathering, sprouting, damage by insects or by microorganisms. The quality characteristics of a pure variety does not change when grown under comparable conditions.

Why did the authors not choose a variety like Tomclair to represent a poor quality durum and a variety like Arcour or Agathe to represent a good quality durum ?
Authors: The aim of this work was not to determine the wheat durum variety giving the best cooking quality or/and the best conditions of manufacturing such spaghetti. The aim of this work was only to correlate, using different and complementary microscopical techniques, the differences in the cooking quality of spaghetti as tested by sensory panels according to some differences observed in the organization of their components. We have chosen the spaghetti manufactured from only one wheat variety to be sure that the structural differences we observed were only due to parameters of production (low or high drying temperature) or to cooking parameters (pH of cooking water).

Apparently no change occurred in the chemical composition of wheat semolina from Montpellier and Niverville but starch granules of sample from Niverville were more damaged, as seen in figure 7, which could be due to environmental effect as weathering. For example, it was shown (Al Saleh and Gallant, 1985) that there is significant difference between the vitreosity values of the same variety of durum wheat cultivated in different geographical regions. As a consequence, there is more chance that starch granules mechanically damaged in milling are due to that factor rather than sprouting or damage by insects or by microorganisms.

J. Jacobs: The drying schedule is not given for HT cycle and should be. Color and flavor can be very negatively affected. Just drying a product is not enough if end-product is unpalatable.
V.L. Youngs: What temperature were used in drying? The effects of VHT drying seem extreme.
R.R. Matsuo: The change in taste and color of the VHT spaghetti suggest a significant denaturation of components brought about by the uncontrolled high temperature.
Authors: Regarding VHT drying, the authors fully recognize that this kind of treatment cannot be used without further studies, particularly in the spaghetti processing. Although certain organoleptic properties such as colour and taste were altered in the VHT spaghetti, this sample showed some exceptional rheological properties. For example, the Index of General Viscoelasticity (IGV) as used by Alary et al. (1979) has also been determined on our samples: wheats Miradur-Niverville and Mondur-Niverville dryed at 40°C and cooked in mineral water presented bad cooking quality and very low IGV, 1.4 and 2.8 respectively; on the contrary, Tomclair-Niverville and Mondur-Montpellier dryed at 40°C and cooked in mineral water presented good cooking quality and higher IGV, 7.9 and 7.7 respectively. Exceptionally, Mondur-Niverville dryed at VHT (higher than 100°C) and cooked in mineral water showed the best cooking quality and the best IGV (better than 10.0). According to these results, the authors thougth that the texture of spaghetti MN-VHT might be bound to a special organization of its components and they decided to study the ultrastructure of one sample presenting such characteristics.

J. Jacobs: Table 1. Difference in cooking quality due to mineral vs distilled water seems extreme.
V.L. Youngs: Are data available on use of distilled water for cooking the MM as well as the MN samples ? The effects of using distilled water on the MN samples seem extreme.
R.R. Matsuo: Why was the sample from Montpellier not also dried at VHT ? Why was the Montpellier sample only cooked in mineral water ? Is the Mondur-Niverville sample cooked in distilled water as good as the Mondur-Montpellier sample cooked in mineral water ?
Authors: VHT drying is unusual. Therefore, it was not applied to all samples.

Cooking in distilled water, as demonstrated by Alary et al. (1979) and D'Egidio et al. (1981) improves the cooking quality of pasta but biochemical mechanisms of such changes are not yet completely known. They could be bound to changes in the acidity or ionic strength. This improvement is more evident when the quality of pasta is poor. For that reason we only cooked the poor quality sample (MN) in distilled water.

Mondur-Niverville (MN) sample cooked in distilled water was slightly stickier than Mondur-Montpellier (MM) sample cooked in mineral water and delitescence was a little bit higher too. Note that pH of cooking water before and after cooking was 7.2 and 9.4 respectively for both MM and MN in mineral water, but only 5.5 and 6.5 respectively for MN in distilled water.

V.L. Youngs: The authors state the poor quality of MN may be related to damaged starch granules that appear. Why is there a difference ? Do the authors have Falling Number data to indicate alpha-amylase activity in the original samples ? This would be useful. Data from other objective measurements also should be included, such as firmness scores, cooking loss.
R.R. Matsuo: The implication of starch damage is questionable. Normal semolina contains a low level of mechanically damaged starch, about 10 Farrnad units, higher if the level of fine particles is higher. If, as the authors claim, the level of damaged starch is high in the MN sample, it must arise from alpha-amylase degradation. If

this is so then the wheat must have been moderately or severely damaged by sprouting. Information on the level of starch damage, amylograph viscosity or Falling Number would be most useful.
Authors: Amyloviscograms of semolina from MN were different according to the water (mineral or distilled) used. In distilled water at 96°C, consistency was higher (438 Brabender Units) than in mineral water (400 BU). But temperature, from which consistency starts to increase, was the same in both cases (87.5 °C).

Solubility of the MN starch granules in boiling water was lower (38.5%) in distilled water than in mineral water (43.6%).

Susceptibility of the starch granules to alpha-amylolysis showed also differences between MM and MN samples. The starch fractions easily digested resulting from the alpha-amylolysis curves was 9 and 13% for MM and MN semolinas respectively.

The starch fraction easily digested in spaghetti was 27.5% for MM, 40.0 and 48.0% for MN, respectively low temperature and VHT.

It is right that Falling Number would have been useful but has not been done.

R.R. Matsuo: No information on commercial sample is given, i.e. whether it was processed by low temperature or high temperature, whether the cooking quality is good or poor.
Authors: We are not commercial and the technology used is usually not labelled on manufactured products. What the microscopists can say concerning spaghetti in figure 1 is that structure of freeze-fractured proteins correspond to a pasta dryed at low temperature.

J. Jacobs: Is not the cooking quality related to quantity of main components ? How about protein quality ? This point is not stressed. There is very limited data to draw such a conclusion.
Authors: Cooking quality is bound to numerous parameters, amongst them the protein rate. Below 10% proteins, characteristics of pasta are generally not good. But between 10 to 15% proteins, characteristics of pasta can be either good or bad. As in our case. Protein quality is also an important factor and is reliable to the variety of the cultivar. The best method to study such parameter is the electrophoretic analysis.

In our study all samples were prepared from the same variety. Electrophoretic outline must be the same for all the samples. But there are probably other parameters, unfortunately unknown (presence of specific groups in proteins) which act and give differences in cooking quality.

J. Jacobs: Extremely long cooking optimum (+ 80%) for HT dried sample makes one wonder about the severity of the drying cycle conditions.
Authors: Longer cooking time for VHT spaghetti is a consequence of modifications which occur during VHT drying, either at the level of the components or at level of their water content.