# Modification of Structure and Digestibility of Chestnut Starch upon Cooking: A Solid State $^{13}C$ CP MAS NMR and Enzymatic Degradation Study

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The modification of starch, which is the major component of the polysaccharide fraction of chestnuts (*Castanea sativa*), has been studied from the point of view of structure and digestibility to understand the modifications induced by cooking and, specifically, by the Maillard reaction. The study was carried out by enzymatic degradation kinetics, monitoring the glucose released upon time, and by solid state $^{13}C$ CP MAS NMR, which has the potential of monitoring the solid state phase changes occurring upon chemical modification due to the cooking process. Results obtained reveal that large changes are induced in the macromolecular structure of starchy materials and that these changes are correlated with changes of digestibility in terms of enzymatic degradation resistance. In the system studied, the extension of the Maillard reaction is not such as to exert a significant influence on structure and/or digestibility of chestnut starch.

**Keywords:** *Chestnuts; starch; starch digestibility; structural modifications*

## INTRODUCTION

Chestnuts (*Castanea sativa*) have an average starch content of 22.3 g/100 g of raw edible portion, whereas, as a comparison, raw potatoes contain 15.9 g/100 g and starch from legumes varies from 19.5 g/100 g (beans) to 2.1 g/100 g (fava beans) (*Italian Food Composition Table*, 1997). This value places chestnuts among the main sources of starch even though the contribution of chestnuts to dietary starch intake, due to their low consumption, is significantly lower than that of potatoes, beans, and legumes.

Starch is the main bioavailable carbohydrate in the human diet. It is digested in vivo, in the small intestine, by excess levels of pancreatic α-amylase, but starch depolymerization can be inhibited by factors intrinsic to starch itself such as cell walls, dense packing structures, and certain crystalline forms of starchy materials and/or by external factors such as the presence of enzyme inhibitors. Furthermore, starch is composed by glucose linked in two different forms, amylose and amylopectin, which are linear and branched polymers of glucose, respectively. Native starch in raw foods has a characteristic crystalline structure depending on the botanical origin of the food. During cooking starch is gelatinized, losing its crystalline organization and, upon cooling, amylose and amylopectin chains reorganize in new crystalline forms. The latter process occurs to a lesser extent due to the disordering effect of the side chains of amylopectin. This different response to retrogradation of amylose and amylopectin also affects the global starch digestibility.

As a result of such interfering factors, a fraction of starch polymers can escape digestion in the small intestine and enter the colon for fermentation. Englyst and collaborators focused on this fraction, called "resistant starch" (RS), and identified two more categories based on the hydrolysis rate: rapidly digestible starch (RDS) and slowly digestible starch (SDS) (Englyst et al., 1992).

Marked changes in digestibility, with respect to enzymatic hydrolysis, have been reported for starch from different botanical species (Pizzoferrato et al., 1995) and for starch subjected to some extent of the Maillard reaction in model systems (Pizzoferrato et al., 1998a). Actually, the Maillard reaction induces changes in the structural features of the glycosidic chain in starch. The solid state NMR spectroscopy indicates that a loss of molecular order, interpreted as the formation of disordered regions that decrease the extent and number of solution crystallites, is due to the occurrence of the Maillard reaction between the reducing end groups of polysaccharides and the free amino groups of proteins. This change in structure correlates with the extent of Maillard reaction as determined by the furosine method, and starch digestibility also decreases markedly as a function of the extent of the Maillard reaction (Pizzoferrato et al., 1998a).

The present paper reports the results of a study on the structural features of chestnut starch upon cooking to ascertain the structural change in starch and the enzymatic hydrolysis dependence on treatments and to shed light on the relationship between the structure and digestibility for these food materials. Also, the possible role of the Maillard reaction between the reducing end of the carbohydrate chains and the free amino groups of proteins has been evaluated. The study was carried out by solid state $^{13}C$ CPMAS NMR, which is able to

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detect the loss of crystallinity in the solid state, particularly in the hydrated form where starch shows a crystalline-like $^{13}C$ NMR spectrum (Torri et al., 1995).

## MATERIALS AND METHODS

**Samples.** Raw chestnuts were purchased from a local supermarket in November 1997 and roasted, in the shell previously cross-cut on the top, on an open fire, according to the traditional Italian recipe. Both raw and cooked chestnuts were hand-peeled and ground by an electric blender.

**Reagents.** All reagents from C. Erba (Milan, Italy) were of analytical or HPLC grade as required. Furosine, 99% purity, was supplied by Neosystem Laboratoire (Strasbourg, France).

Hydrolytic enzymes were purchased from Sigma Chemical Co. (St. Louis, MO). The glucose colorimetric kit was a glucose GOD-PAP supplied by Boehringer Mannheim (Milan, Italy).

**Apparatus.** $^{13}C$ CPMAS NMR spectroscopy was performed by a Bruker instrument AM 400 operating at 100.56 MHz in the magic angle cross-polarization mode. The contact time between proton and carbon magnetization was 1.2 ms and the number of scans 1024.

The HPLC analytical system, used for furosine determination, included a Waters (Milford, MA) Model 510 solvent delivery system, a Gilson (Middleton, WI) Model 231-401 autosampling injector, and a Waters (Norwalk, CT) Model 490 programmable multiwavelength spectrophotometer.

**Procedures.** The digestibility of chestnut starch was tested at 37 °C, in 0.2 M sodium acetate buffer (pH 5), using pancreatin and amyloglucosidase (EC 3.2.1.3) as hydrolytic enzymes. This method is based on an in vitro model of the physiological starch digestion in man and agrees favorably with the in vivo assessments of starch digestibility (Englyst et al., 1992). An amount of ground chestnuts, estimated to contain nearly 0.5 g of starch, was incubated with pancreatin (250 mg) and amyloglucosidase (70 AGU). Samples were withdrawn at various times (from 0 to 120 min), and the amount of D-glucose released upon hydrolysis was measured by a colorimetric test. During this analytical procedure, the enzyme glucose oxidase (GOD) catalyzes the oxidation of glucose in gluconic acid. The hydrogen peroxide formed during this reaction reacts with 4-aminoantipyrine and 4-hydroxybenzoic acid in the presence of peroxidase (POD) to form *N*-4-antipyryl-*p*-benzoquinone imine readable at 510 nm.

The amount of starch degraded after 20 and 120 min of incubation with the enzyme mixture used in the Englyst method corresponds to the RDS fraction and to the SDS fraction, respectively. The undigested starch residue is the RS fraction.

Amylose content was determined, after isolation of chestnut starch and precipitation with *n*-butanol, according to the method of McCracken and Cain (1981).

The occurrence of the Maillard reaction were determined by HPLC (Pizzoferrato et al., 1998b) by analyzing the level of furosine. This analytical artifact, liberated by acid hydrolysis of each sample, can be used as an indicator of the early stage of the Maillard reaction (Erbersdobler and Hupe, 1991). A weighted aliquot of sample was hydrolyzed by HCl (6 M) at 110 °C for 24 h, and the furosine amount was detected at 280 nm after a chromatographic elution with 60 mM sodium acetate buffer at pH 3.0 using a 5 $\mu$m, 250 × 4.6 mm i.d., Supelcosil LC-8 column (Supelco Inc., Bellefonte, PA).

Each analysis was performed in three replicates.

## RESULTS AND DISCUSSION

The change of structure of chestnut starch upon cooking has been tested by $^{13}C$ CPMAS NMR spectroscopy. Figure 1 reports the $^{13}C$ NMR spectrum in the solid state of the chestnut as purchased (A) and after cooking as detailed under Materials and Methods (B).

As is known, the solid state NMR spectrum reflects the fact that every molecule has a magnetic environment which repeats regularly to originate a rather narrow dispersion of chemical shifts. In the presence of other types of molecular arrangements and/or other crystalline phases or molecular disorder, the shape of the resonances changes markedly. Thus, a drastic decrease of the intensity of some NMR signals and the increase of other signals must be attributed to the loss of molecular order or generally of crystallinity.

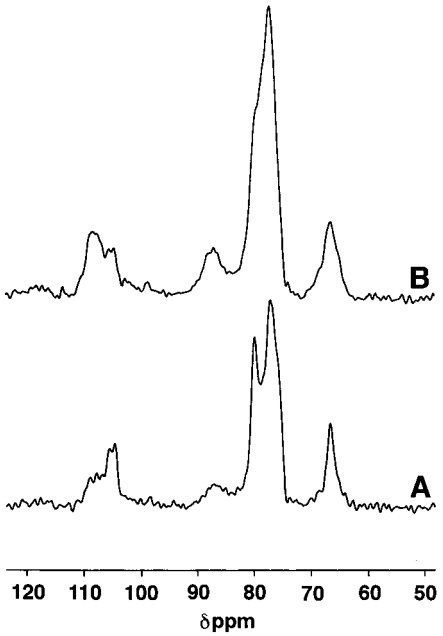


**Figure 1.** Solid state $^{13}C$ NMR CPMAS spectrum of the chestnut as purchased (A) and after roasting (B) as reported under Samples. Only part of the spectrum is displayed. The spectrum has been obtained as reported under Apparatus, and data were treated with a Gaussian−Lorentzian multiplication before FT transformation to enhance resolution. The assignments of the protonated carbons were as follows: C1, 103−108 ppm; C4, 85−90 ppm; C2, C3, C5, 74−82 ppm; C6, 68 ppm.

The shape of the C-1 carbon resonances of the hydrocarbon chains of both amylose and amylopectine is particularly sensitive to structural features in the crystalline state. These resonances, ∼103−108 ppm, show a clear difference in shape (Figure 1A,B). They are due to envelopes containing different resonances caused by both amylose and amylopectin in markedly different crystalline states (allomorphs). A change in the intensity of some components leads to a meaningful change in shape, and it is a clear indication that the component composition changed. Particularly amylose, which is present in chestnut starch in high levels [48% in the sample studied and nearly 45% in the literature (Se Kwon et al., 1995)], is shown to be in two well-characterized allomorphs B and V. In the B form, the starch α-glucan chains exist as left-handed, parallel-stranded double helices and the center of the array is occupied by water. This pattern is found in potato, banana, and high-amylose maize starches and is particularly resistant to digestion (Brown, 1996). When suitable "guest" molecules such as lipids or ions are present, segments of the amylose chain have the ability to form single left-handed V-type helices with a hydrophobic cavity where the guest molecule is included in a clathrate complex (Sivak and Preiss, 1998). The resonances at 103 and 108 ppm can thus be reasonably attributed to the relevant extent of these allomorphs: in the roasted sample a decrease of the B form (103 ppm) and an increase of the V form (108 ppm) can be

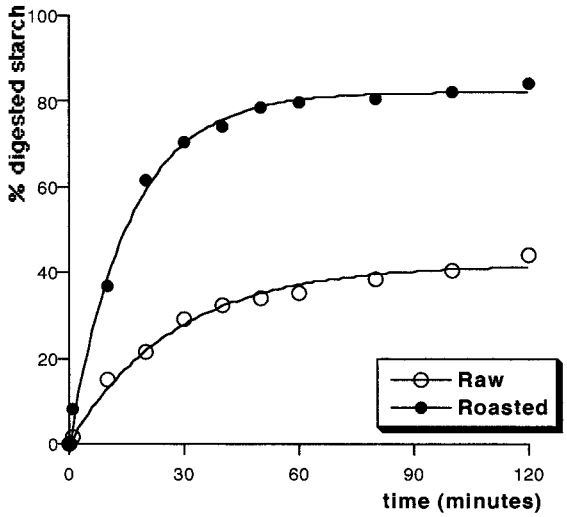


**Figure 2.** Kinetics of starch digestibility in chestnuts as purchased (○) and after roasting (●). Experimental conditions are reported under Procedures.

**Table 1. Effect of Cooking on the Digestibility of Starch in Chesnuts**[a]

| | g/100 g of starch | | |
|---|---|---|---|
| chestnuts | RDS | SDS | RS |
| raw | 21.52 ± 1.21 | 20.91 ± 1.69 | 57.56 ± 1.54 |
| roasted | 60.29 ± 2.21 | 22.80 ± 1.17 | 16.91 ± 1.07 |

[a] Values of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) are means of three different digestion tests.

observed if compared with the raw sample. This feature indicates that long and ordered crystallites are still present in the cooked samples but a marked modification of the high-field component of the resonance envelope is distinguishable. Taking into account the results of previous work (Horii et al., 1987), this type of shape is assigned to a prevalence of the single-chain glucidic moiety in the crystallites that should be more accessible to the enzymatic attack (Torri et al., 1995).

Enzymatic hydrolysis reported in Figure 2 clearly shows that the two chestnut samples have very different accessibilities to the action of specific enzymes. Depending on whether they were previously cooked or not, the kinetics of glucose release from the polymeric chain occurs with very different speeds in the two samples. On the basis of these results, the evaluation of the three starch fractions was performed. The relevant results are reported in Table 1 and show that the main differences are in the RDS and in the RS fractions. The cooking procedure causes a considerable increase in the RDS fraction, from 21 to 60% of total starch, and a consequent decrease in the RS fraction from 58 to 17%. The SDS fractions show no significant differences ($p \geq 0.05$) between the raw and roasted chestnut samples. These results indicate that a drastic modification occurred in the chestnut during heating.

Taking into account the above-reported NMR spectra, a correlation can be observed between the rate of the enzymatic action and the structure of the crystallites of the starch chain: in fact, the single-helix allomorph (V form), as already reported (Torri et al., 1995), shows higher digestibility and higher accessibility to the enzyme action than the other allomorphs.

Furthermore, in a previous study (Pizzoferrato et al., 1998a), changes in the structural features of the glycosidic chain in starch indicating loss of molecular order were correlated with the extent of the Maillard reaction. To verify the occurrence and the extent of the Maillard reaction, in this case, furosine was prepared and chromatographically analyzed. After the chestnut raw starch had been cooked, only a negligible amount of furosine was found in the samples. These results indicate that the Maillard reaction does not influence significantly the digestibility of chestnut starch.

Probably in this experiment the modification in the starch digestibility is essentially due to the effect of the heat treatment on the structural arrangement of the starch macromolecule even if an indirect effect on natural and thermolabile α-amylase inhibitors cannot be excluded. This point will be further clarified and is presently under study.

## CONCLUSIONS

The solid state NMR spectroscopy indicates that no dramatic loss of molecular order, interpreted as the formation of disordered regions that decrease the extent and the number of solution crystallites, occurs for starch from chestnut flour upon cooking. A large rearrangement of the starch components toward a different distribution of conformation is visible. Particularly, a predominance of single-chain aggregates was observed in the $^{13}C$ CP MAS spectrum, and this finding is likely correlated with the increase of starch digestibility as shown in the enzymatic digestion test.

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