Structural Modification and Bioavailability of Starch Components As Related to the Extent of Maillard Reaction: An Enzymatic Degradation and a Solid-State ^{13}C CPMAS NMR Study

Laura Pizzoferrato,*,† Maurizio Paci,‡ and Giuseppe Rotilio†,§

National Institute of Nutrition, Rome, Italy, Department of Chemical Science and Technology, Tor Vergata University, Rome, Italy, and Department of Biology, Tor Vergata University, Rome, Italy

Starch and starch components, amylose and amylopectin, from potato, have been studied in order to understand the modifications, induced by different levels of the Maillard reaction occurring in the presence of amino acids (L-lysine in this study), in the macromolecular structure and digestibility. Structural characterization was performed by ^{13}C CPMAS NMR, and differences in the bioavailability of these polymers were studied by enzymatic degradation kinetics. Results obtained reveal that changes are induced by the Maillard reaction, whose occurrence has been verified and measured by the furosine (ϵ-*N*-2-furoylmethyl-L-lysine) evaluation, on the macromolecular structure of starchy materials and on their enzymatic susceptibility. Lysine seems to act as a disordering agent; a loss of crystallinity is evident for starch and amylopectin and, in particular, for amylose. Finally, within the same sample, as the lysine concentration increases, the polymer digestibility decreases, confirming that structural organization is not the only determinant of starch digestibility.

Keywords: *Starch components; Maillard reaction; starch digestibility; structural modifications*

INTRODUCTION

Starch represents the major source of carbohydrates in the human diet. In vivo starch is digested in the small intestine by pancreatic α-amylase, but starch hydrolysis can be inhibited by intrinsic factors 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.

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. Recent studies (Englyst et al., 1992) have focused on this fraction, called "resistant starch" (RS), and two categories are based on the hydrolysis rate: rapidly digestible starch (RDS) and slowly digestible starch (SDS). Furthermore, starch consists of D-glucose assembled in two major forms: amylose and amylopectin; the former one is a linear polymer, while the latter one is a branched polymer. Amylose and amylopectin contents, due to the different native structure and to a different response to the arrangement after heating of unwieldy molecules into crystalline structures (retrogradation), can also affect the global starch digestibility.

Cooking of food, such as bread or pasta, may also favor the occurrence of chemical events such as the Maillard reaction (Friedman, 1996). The extent of this reaction in the presence of proteinaceous materials, particularly of lysine residues, lowers the bioavailability of lysine in the diet and has been extensively studied in the past (Mauron, 1981). The reaction occurs between the side-chain amino groups of lysine and the carbonyl group of reducing sugars and lysine is converted to a nonbioavailable N-substituted form called the Amadori compound. By acid hydrolysis of the Amadori compound, furosine (ϵ-*N*-2-furoylmethyl-L-lysine) is formed in a fixed amount (32% of the blocked lysine). This analytical artifact can be used as an indicator of the early stage of the Maillard reaction (Erbersdobler and Hupe, 1991).

The inhibition of α-amylase and proteases by melanoidin, a brown product from the advanced stage of the Maillard reaction, has been already reported (Miura and Gomyo, 1993; Hirano et al., 1996). The aim of this work is to clarify to what extent this reaction, in a very early stage, may affect the digestibility of starch and starch components, amylose and amylopectin, modifying the structural features. The study was carried out by solid-state ^{13}C CPMAS NMR, which is able to detect the loss of crystallinity in the solid state, particularly in the hydrated form, where starch and its components, instead of a solution, show a crystalline-like ^{13}C NMR spectrum.

This study in particular focused on (i) the change in macromolecules of model systems containing starch (or amylose or amylopectin) and different amounts of lysine residues, treated at controlled temperature and water content and simulating the cooking of starch in the presence of proteinaceous material, and (ii) the occurrence of the Maillard reaction under the conditions studied and the effect of both heat treatments and lysine bonds on the macromolecule digestibility.

MATERIALS AND METHODS

Apparatus. ^{13}C CPMAS NMR spectroscopy was performed with a Bruker AM 400 operating at 100.56 MHz in the magic-angle cross-polarization mode. The contact time between

* Address correspondence to this author at the Istituto Nazionale della Nutrizione, via Ardeatina 546, 00178 Roma, Italy (fax, 39.6.5031592; e-mail pizzof@inn.ingrm.it).
† National Institute of Nutrition.
‡ Department of Chemical Science and Technology.
§ Department of Biology.

S0021-8561(97)00713-9 CCC: $15.00
Published on Web 01/27/1998

proton and carbon magnetization was 1.2 ms and the number of scans 1024.

The HPLC analytical system used included a Waters (Milford, MA) Model 510 solvent delivery system and a Gilson (Middleton, WI) Model 231-401 autosampling injector, and a Waters (Norwalk, CT) Model 490 programmable multiwavelength spectrophotometer was utilized. Furosine chromatographic separation was performed using a 5 μm, 250 $\times$ 4.6 mm i.d., Supelcosil LC-8 column (Supelco Inc., Bellefonte, PA).

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).

Amylopectin from potato, amylose from potato (type III essentially free of amylopectin), potato starch, and hydrolytic enzymes were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose colorimetric kit was a Glucose GOD-PAP supplied by Boehringer Mannheim (Milan, Italy).

Procedures. Experiments were carried out by heating at 100 °C for 45 min mixtures of lysine solution with amylose or amylopectin or starch at different ratios to obtain solid samples which have been tested for ^{13}C CPMAS NMR. Taking into account the water-holding capacity of each polymer, 2 mL of lysine solution (5, 50, and 150 mM) was added to 0.125 g of amylose, 1 g of amylopelctin, and 0.625 g of starch.

The occurrence of the Maillard reaction and the level of carbonyl-linked lysine were determined by HPLC (Pizzoferrato et al., 1997) by analyzing the furosine liberated by acid hydrolysis of each sample (Resmini et al., 1990). A weighted aliquot of sample was hydrolyzed by HCl (6 M) at 110 °C for 24 h; furosine amount was detected at 280 nm after a chromatographic elution with 60 mM sodium acetate buffer at pH 3.2.

The digestibility of starch and starch components was tested at 37 °C in 0.2 M sodium acetate buffer (pH 5) using pancreatin and glucoamylase (EC 3.2.1.3) as hydrolytic enzymes. 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. The 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).

RESULTS AND DISCUSSION

The decrease of crystallinity as a function of the extent of the Maillard reaction has been tested by ^{13}C CPMAS NMR spectroscopy of amylose, amylopectin, and starch in the presence of different amounts of lysine upon heating at 100 °C for 45 min. The results are reported in Figure 1. With increasing lysine concentration, the crystallinity degree of amylose, amylopectin, and potato starch, composed of 20% amylose and 80% amylopectin (Kearsley and Sicard, 1992), decreases as tested by the relevant decrease of the resonance signals in the ^{13}C CPMAS NMR spectra.

The solid-state NMR spectrum reflects the fact that every molecule has a magnetic environment which repeats regularly so as to produce a rather narrow dispersion of chemical shifts. On the contrary, in the presence of molecular disorder, the dispersion is larger due to the large number of environments and, moreover, variable with time. Thus a drastic decrease of the intensity of NMR signal under these conditions is to be attributed to the loss of molecular order or, generally, crystallinity.

In the case of starch and starch components, the C-13 resonances of the D-glucose moiety, upon hydration, give rise to sharp resonances and formation of large molecular crystallites due to interactions with water (Horii et al., 1987; Torri et al., 1995). The presence of water in starch and starch components induces a transition

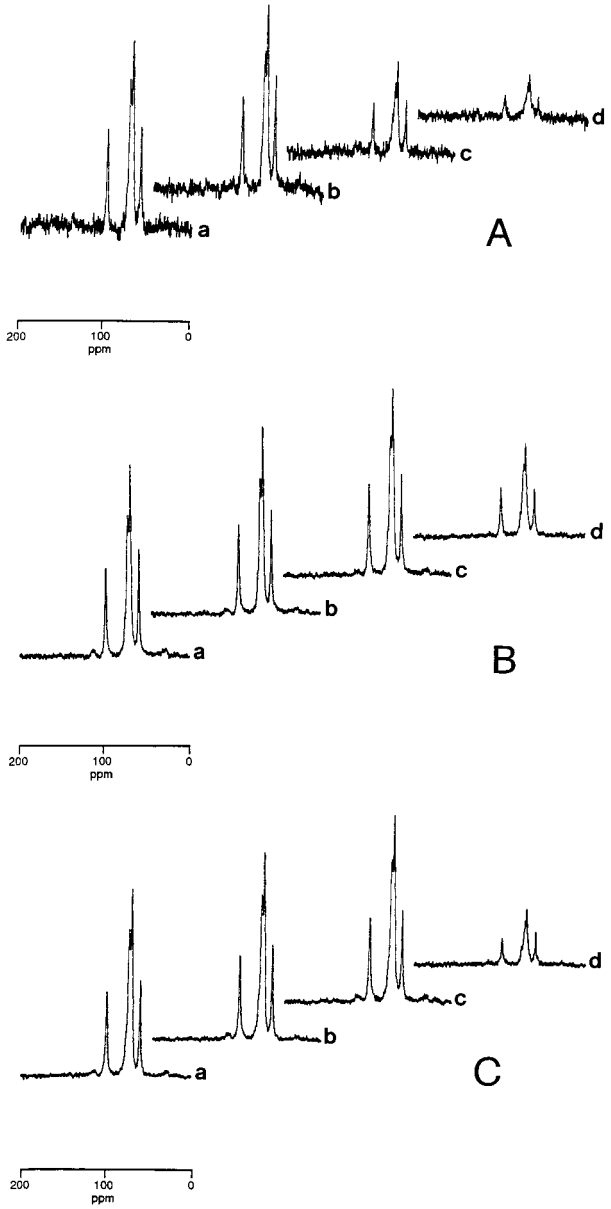

Figure 1. ^{13}C CPMAS high-resolution solid-state NMR spectra of (A) amylose, (B) starch, and (C) amylopectin from potato as related to the extent of Maillard reaction with lysine at different concentration (a = 0 mM, b = 5 mM, c = 50 mM, and d = 150 mM). Experimental conditions are reported under Materials and Methods.

toward crystallinity, contrary to what happens in a soaked solid where usually molecular disorder is induced by water. Deprivation of water leads to the formation of amorphous fractions which display very broad resonances, easily lost in the experimental noise.

In our case the decrease of the ^{13}C NMR resonances in the solid state can be attributed to this loss of crystallinity. In particular, the decrease of the normalized intensity of the resonance attributed to the C-1 carbon of glucose polymer can be used to monitor the structural changes.

It can easily be seen that the effect of the reaction is different in the three cases. It is less pronounced in the case of amylopectin and starch. In fact, in the same conditions and at the same lysine content, the loss of intensity (i.e., the loss of crystallinity) is more pronounced for amylose than for the others.

The evidence that no other resonances appear in the

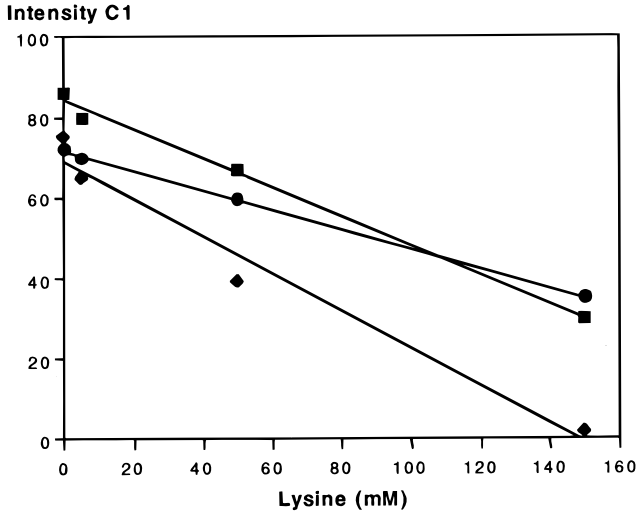

Figure 2. Intensity of the C1 resonance in (♦) amylose, (■) amylopectin, and (●) starch treated at 100 °C for 45 min vs lysine concentration. Experimental conditions are reported under Materials and Methods.

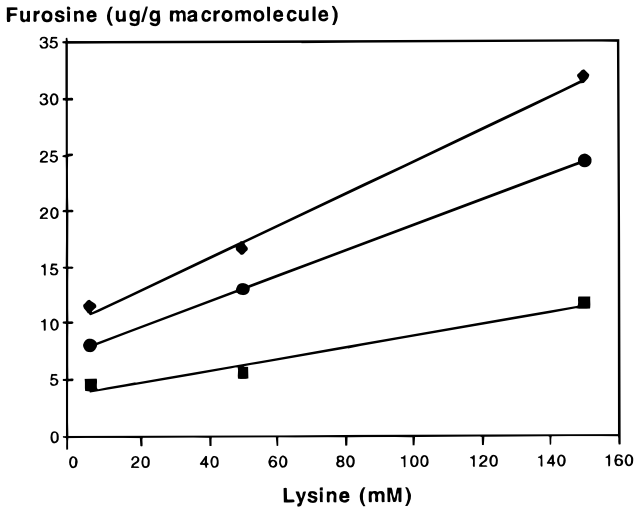

Figure 3. Furosine contents of (◆) amylose, (■) amylopectin, and (●) starch treated at 100 °C for 45 min vs lysine concentration. Experimental conditions are reported under Materials and Methods.

spectrum also in the presence of high concentration of lysine confirms this interpretation. In fact, reaction between lysine and the reducing end acts as a disordering agent in the soaked solid, thus leading to the disappearance of the resonances of the carbohydrate polymer together with those belonging to the lysine reacted. Unreacted lysine is in solution and, thus, is disordered.

To monitor these changes in Figure 2 the intensities of the resonances due to the C-1 carbon of the D-glucose monomeric unit (at 105 ppm) are reported as a function of the lysine concentration. For lysine concentrations ranging between 0 and 150 mM lysine, a good linear correlation between these parameters can be observed: $r = 0.99$ (amylose) or 1.00 (amylopectin and starch). As already described, amylose shows the largest reactivity followed by amylopectin. Starch has an intermediate behavior similar to that of amylose at low lysine levels and that of amylopectin at high lysine levels.

These results indicate that the difference in reactivity induces differences in the crystal structure. Particularly, amylose crystallinity decreases more drastically than amylopectin crystallinity (line negative slope = 0.46 and 0.36, respectively) and at lower lysine concentration. The combination of amylose and amylopectin leads to a polymer mixture (starch) which is more resistant to the loss of crystallinity induced by the reaction with lysine (line negative slope = 0.24).

The number of reducing end groups seems to be the rationale for this behavior. In fact, amylopectin and amylose both have one reducing end per mole, but due to the greater molecular weight, a fixed mass of amylopectin has a lower number of reducing ends than the same mass of amylose.

The reducing end should be the site of the lysine attack during the early stage of the Maillard reaction, and the occurrence of this reaction can be monitored by furosine analysis. Furosine is an analytical artifact formed by acid hydrolysis of the Amadori compound and can be used as an indicator of the Maillard reaction. Furosine contents of the studied samples are reported in Figure 3 as a function of the lysine concentration. The correlation among the results is quite good and the correlation factor is $r = 1.00$ in starch and amylose and $r = 0.99$ in amylopectin. Furthermore, confirming the significance of the number of reducing ends, amylose shows the highest and amylopectin the lowest furosine level, while again starch has an intermediate behavior. The different reactivities can be also evaluated from the line slope values of the linear equation fitting the experimental data: 0.14 (amylose), 0.05 (amylopectin), and 0.11 (starch).

According to previous studies, polymeric sugars should be thermally degraded to monomers or at least to dimers before the Maillard reaction can occur (Kroh et al., 1997). However, the relatively mild heat treatment (100 °C) and the absence of detectable levels of free D-glucose in the studied samples strongly suggest that no meaningful thermolysis occurred in the described model system. Moreover, potato starch is reported to give sugars only when heated at 200−250 °C (Tomasik et al., 1989).

Finally, in Figure 4, the D-glucose released upon enzymatic hydrolysis for 60 min is reported as the percentage of the D-glucose released at the same time by the corresponding control sample without lysine addition.

Untreated amylose, amylopectin, and starch show the highest digestibility at 60 min, and the digestibility decreases after heat treatment. In fact, retrograded starch, one of the forms of resistant starch, refers to the aggregates that are formed by cooling after cooking of starches. The linear amylose is reported to retrograde more rapidly than the branched amylopectin polymers (Schulz et al., 1993), and that is confirmed by the lowest hydrolyzed D-glucose levels. However, amylopectin also undergoes retrogadation even with a lower efficiency, playing a role in decreasing digestibility (Pizzoferrato et al., 1995), and this is also evident in Figure 4.

Within the same sample, as the lysine concentration increases, the digestibility decreases, which seems to be surprising since amorphous polymers are usually more digestible than crystalline ones. Moreover, the digestibility slightly decreases, as the lysine level increases, even in untreated samples where the furosine content is zero and the Maillard reaction has not taken place. In the latter case the decrease is slower (line negative slope = 0.02−0.07) than in the treated samples (line negative slope = 0.10−0.18), but significant.

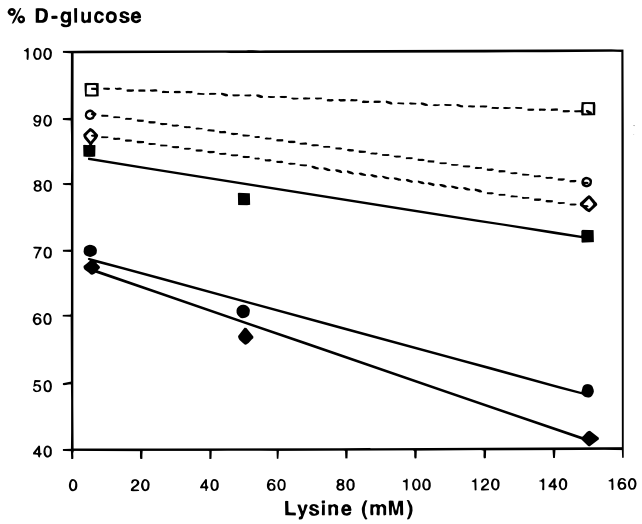

Figure 4. D-Glucose hydrolyzed at 60 min vs lysine concentration. The released D-glucose is expressed as percentage of the D-glucose released in the same condition by the control sample without lysine addition. Treated (100 °C for 45 min) (◆) amylose, (■) amylopectin, and (●) starch and untreated (◇) amylose, (□) amylopectin, and (○) starch. Experimental conditions are reported under Materials and Methods.

Probably the Maillard reaction and the loss in crystallinity are not the only determinants in these experiments, and an effect of lysine charge on the structural organization and on the enzyme activity cannot be ruled out.

CONCLUSIONS

Maillard reaction induces changes in the structural features of the glycosidic chain in both amylose and amylopectin and also in starch which is the natural mixture of them.

Solid-state NMR spectroscopy indicates that a loss of molecular order, interpreted as the formation of disordered regions which decrease the extent and the number of solution crystallites, is due to Maillard reaction occurring between the reducing end groups of polysaccharides and the amino groups of lysine. The change in structure correlates with the extent of Maillard reaction as determined by the furosine method. These results reveal that the modification is much more drastic in the case of amylose than for amylopectin and starch. The Maillard reaction, however, contributes to decreasing amylose, amylopectin, and starch digestibility as shown in the enzymatic digestion test.

ACKNOWLEDGMENT

For assistance and execution of solid state NMR, Fabio Bertocchi is gratefully acknowledged.

LITERATURE CITED

Englyst, H. N.; Kingman, S. M.; Cummings, J. K. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* **1992**, *46* (Suppl. 2), S33−S50.

Erbersdobler, H. F.; Hupe, A. Determination of lysine damage and calculation of lysine bio-availability in several processed foods. *Z. Ernaehrungswiss.* **1991**, *30*, 46−49.

Friedman, M. The impact of the Maillard reaction on the nutritional value of food proteins. In *The Maillard reaction. Consequences for the chemical and life sciences*; Ikan, R., Ed.; Wiley: Chichester, U.K., 1996; Chapter 5.

Hirano, M.; Miura, M.; Gomyo, T. Kinetic analysis of the inhibition by melanoidin of trypsin. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 458−462.

Horii, F.; Yamamoto, H.; Hirai, A.; Kitamaru, R. Structural study of amylose polymorphs by cross-polarization-magic-angle-spinnin ^{13}C-NMR spectroscopy. *Carbohydr. Res.* **1987**, *160*, 29−40.

Kearsley, M. W.; Sicard, P. J. The chemistry of starches and sugars present in food. In *Dietary Starches and Sugars in Man: A Comparison*; Dobbing, J., Ed.; Springer-Verlag: London, 1992; Chapter 1.

Kroh, L. W.; Haseler, J.; Hollnagel, A. Role of oligo and polymeric carbohydrates in the Maillard reaction. *Abstracts of Papers*, The 6th International Symposium of The Maillard Reaction; Royal College of Physicians: London, 1997; p 134.

Mauron, J. The Maillard reaction in food: a critical review from the nutritional standpoint. *Prog. Food Sci. Nutr.* **1981**, *5*, 5−35.

Miura, M.; Gomyo, T. Effect of the brown pigment melanoidin on digestive enzymes in human saliva. *J. Jpn. Soc. Nutr. Food Sci.* **1993**, *46*, 309−316.

Pizzoferrato, L.; Aguzzi, A.; Cappelloni, M.; Bonmassar, L. Studies on *in vitro* digestibility of starches from different botanical sources. In *Atti del II Congresso Nazionale di Chimica degli Alimenti*; La Grafica: Messina, Italy, 1995; Vol. II, pp 1129−1134.

Pizzoferrato, L.; Manzi, P.; Vivanti, V.; Nicoletti, I.; Corradini, C.; Cogliandro, E. Maillard reaction in milk-based foods: nutritional consequences. *J. Food Prot.* **1997**, in press.

Resmini, P.; Pellegrino, L.; Battelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.* **1990**, *2*, 172−183.

Schulz, A. G. M.; Van Amelsvoort, J. M. M.; Beynen, A. C. Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. *J. Nutr.* **1993**, *123*, 1724−1731.

Tomasik, P; Wiejak, S.; Palasinki, M. The thermal decomposition of carbohydrates. Part II. The decomposition of starch. In *Advances in Carbohydrate Chemistry and Biochemistry*; Tipson, R. S., Horton, D., Eds.; Academic Press: San Diego, CA, 1989; Vol. 47, pp 279−343.

Torri, G.; Naggi, A.; Cosentino, C.; Pizzoferrato, L.; Aguzzi, A.; Cappelloni, M. Relationship between starch structure and *in vitro* digestibility: a preliminary study. in *Atti del II Congresso Nazionale di Chimica degli Alimenti*; La Grafica: Messina, Italy, 1995; Vol. II, pp 1121−1127.

Received for review August 18, 1997. Revised manuscript received October 28, 1997. Accepted November 5, 1997.[⊗]

JF970713+

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1997.