

Table IV. Potential Yields from Jerusalem Artichoke Herbage Harvested at One-Month Intervals (Yield for Three Cuttings)

constituent	potential yield, kg/ha
dry matter	26956
protein	5392
protein in isolate at 15% recovery	808
lysine recovered in isolate	48

such as alfalfa, sorghum, corn, etc. The recovery of protein as an isolate of about 800 kg/ha represents a fraction of the crude protein of the herbage in a highly concentrated form. It appears to store well at room temperature and may be used for a variety of purposes, especially for diet enrichment.

The crude protein content was not enhanced greatly by the nitrate level in the case of early cuttings of Jerusalem artichoke herbage. We have found much higher nitrate levels when immature plants of other species are harvested (corn, sorghum, sudan grass, oats, etc.). In our test plots there was only a slight decrease in tuber yield attributable to harvesting of immature forage. These early cuttings were made well before the tubers began to develop. Such utilization of Jerusalem artichoke herbage may make this crop more attractive to the producer as well as to the processor.

Registry No. Phosphorus, 7723-14-0; calcium, 7440-70-2; magnesium, 7439-95-4; zinc, 7440-66-6; iron, 7439-89-6; nitrate, 14797-55-8.

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Received for review June 11, 1984. Accepted October 12, 1984. Partially reported at the 17th American Chemical Society Great Lakes Regional Meeting, St. Paul, MN, June 1-3, 1983. Published as Paper No. 7499, Journal Series, Nebraska Agricultural Experiment Station.

Effects of Temperature on Maillard Reaction Products

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Equimolecular amounts of D-xylose and glycine were reacted in aqueous solution at temperatures of 22, 68, and 100 °C. Infrared, cross polarization-magic angle spinning ¹³C NMR of the high molecular weight melanoidins, and ¹³C solution spectra of the low molecular weight soluble products showed an increase in aromaticity with reaction temperature. The structure of the melanoidin synthesized at 22 °C differed considerably from those synthesized at higher temperature: different types of aliphatic carbons and fewer unsaturated carbons.

Temperature, time, moisture content, concentration, and nature of reactants are important factors in the Maillard reaction (Gomyo et al., 1972; Nursten, 1980-1981; Vernin, 1980). It has been reported that browning increased exponentially with temperature (Shallenberger and Birch, 1975; Vernin, 1980) and that heating melanoidins, the dark brown nitrogenous polymers formed in the reaction, for long periods, causes discoloration and fragmentation (Gomyo et al., 1972). Bobbio et al. (1981) reported the microanalytical data of melanoidins synthesized from glucose and glycine at 70, 80, and 90 °C. Little is known, however, on the effects of temperature on the chemical structure of the Maillard reaction products.

Recently it was shown (Benzing-Purdie and Ripmeester, 1983; Benzing-Purdie et al., 1983), with solid-state ¹³C and ¹⁵N NMR, that remarkable similarities exist between humic substances and melanoidins synthesized from car-

bohydrates and amino acids at 68 °C. Since the latter temperature is high by soil standards, it was essential to complement the work by further studying the temperature effects on the products of the Maillard reaction. The present communication compares the chemical structures of the high molecular weight melanoidins and the low molecular weight products obtained from the reaction of xylose and glycine at three different temperatures 22, 68, and 100 °C.

EXPERIMENTAL SECTION

Chemicals. D-Xylose was purchased from Pfanziehl Laboratories, Inc., Waukegan, IL 60085, and glycine from Sigma Chemical Co., St. Louis, MO 61378.

Synthesis of Melanoidins. *Synthesis of Melanoidin at 22 °C (Am).* A sterile solution of D-xylose (1.5 g) and glycine (0.75 g) in water (10 mL) was kept under sterile conditions at 22 °C over a period of 9 months. A duplicate experiment was run in presence of NaNO₂ (0.02%). The melanoidins were recovered after exhaustive dialysis in tubing with a molecular weight cutoff of 12000, evaporated at 68 °C, and dried over P₂O₅ to give Am: yield, 0.095 g; partial IR 1730 (vw), 1630 (s), 1500 cm⁻¹ (vw). Anal.

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Found: C, 50.29; H, 5.32; N, 7.98. No difference in yield or physical properties of the melanoidins synthesized in the presence or absence of NaN_2 was noticed.

Synthesis of Melanoidin at 68 °C (Bm). A sterile solution of D-xylose (15 g) and glycine (7.5 g) in water (100 mL) was kept under sterile conditions at 68 °C. At the end of 6 weeks the precipitate was filtered, washed until the water effluent was colorless, and dried over P_2O_5 to give Bm: yield, 8.9 g; partial IR 1710 (w), 1630 (s), 1550 cm^{-1} (w). Anal. Found: C, 57.05; H, 6.08; N, 7.33. The filtrate and washings were combined, evaporated under reduced pressure, and dried over P_2O_5 to give a light brown amorphous, hygroscopic solid, Bs: yield, 6.75 g.

Synthesis of Melanoidin at 100 °C (Cm). A sterile solution of D-xylose (15 g) and glycine (7.5 g) in water (100 mL) was refluxed. After 38 h, the reaction mixture was filtered, and the precipitate was washed until the effluent was colorless and dried over P_2O_5 to give Cm: yield, 6.91 g; partial IR 1710 (w), 1630 (s), 1550 cm^{-1} (w). Anal. Found: C, 57.79; H, 5.37; N, 6.68. The filtrate and washings were combined, evaporated under reduced pressure, and dried over P_2O_5 to give Cs: yield, 3.55 g of a light brown amorphous hygroscopic solid.

Cross Polarization-Magic Angle Spinning (CP-MAS) ^{13}C NMR Spectra. The spectra were obtained at 45.28 MHz on a Bruker CXP-180 pulsed spectrometer with cross polarization and magic angle spinning as previously reported (Benzing-Purdie and Ripmeester, 1983): cross polarization time, 1 ms; time between successive scans, 2 s; number of scans, 3000–6000.

Proton Decoupled ^{13}C Solution Spectra. The solution spectra were obtained on a Bruker WM-250 spectrometer using 0.30 g of soluble product in 2 mL of D_2O , in a 10-mL NMR tube. Continuous proton decoupling, a sweep width of 15 000 Hz, a 90° pulse, and a delay of 10 s were used as operating conditions: NS, 1200 (Bs); NS, 7800 (Cs).

Infrared Spectra. The spectra were recorded on a Beckman 4250 spectrometer using KBr disks.

RESULTS AND DISCUSSION

The reaction of D-xylose and glycine has been studied earlier (Maillard, 1916; Anet, 1959; Miller et al., 1980; Masayo and Toshiharu, 1982). With the exception of Maillard, who reported the results of a study based on a visual examination of the browning rate of this reaction at temperatures of 34, 40, and 100 °C, no other work on the specific effects of temperature on the chemical structures of the products formed has been published.

Shallenberger and Birch (1975) reported an interaction between the different factors affecting the Maillard reaction; time, temperature, nature of reactants, concentration, and moisture content. A study on the effects of temperature on the Maillard reaction products is dependent on reaction time. Kinetic studies have been reported on the temperature dependence of this reaction (Labuza and Saltmarsh, 1981). The terms Q_{10} , defining the increase in rate for every 10 °C increase in temperature, has been shown to range from 2 to 8. It was, therefore, not feasible in the present study to maintain the time constant, because of the large temperature difference. When equimolecular amounts of carbohydrates and amino acids were employed, the carbohydrate is consumed much faster than the amino acid. At higher temperatures, therefore, the disappearance of xylose was used to monitor the progress of the reaction. The reactions carried out at 68 and 100 °C were stopped after 6 weeks and 38 h, respectively, when only trace amounts of xylose could be detected in NMR spectrum (Figure 1). The reaction done at 22 °C was terminated

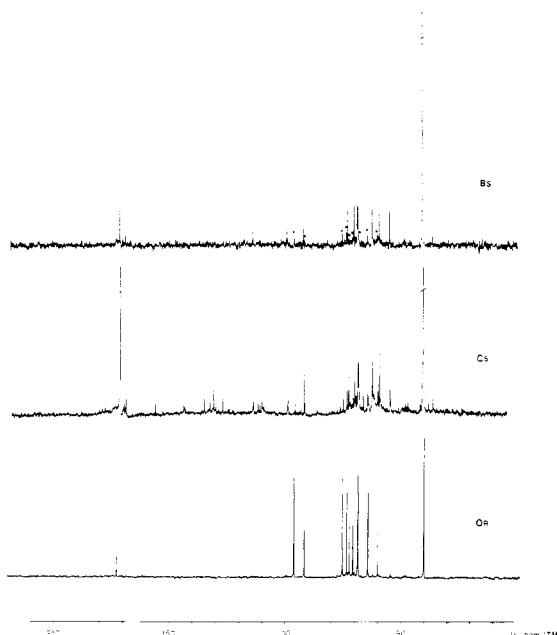


Figure 1. ^{13}C solution spectra of Cs, Bs, and Or: the soluble products obtained at 100, 68 °C, and the starting materials, respectively. (*) Residual xylose.

after 9 months although large amounts of both starting materials were still present in the reaction mixture. The amounts of both melanoidin and soluble material were greater at 68 °C than at 100 °C, with a total recovery of 70% and 46%, respectively. This difference may be attributed mainly to the formation of more volatile products at higher temperatures. The formation of numerous volatile products in this reaction is well documented (Shallenberger and Birch, 1975; Vernin et al., 1983). The loss of 30% of the material at 68 °C is most likely due in part to the formation of CO_2 and H_2O . The yield of the melanoidin Am, synthesized at 22 °C, is low (4%). Microanalytical data show that the latter substance differs greatly in composition from the melanoidins Bm and Cm, synthesized at 68 and 100 °C, respectively; the C content is much lower while the N content is higher. These results imply a higher oxygen content and therefore a lesser degree of dehydration. In other words, less unsaturation. The difference between Bm and Cm is less but noticeable. The C content increases with temperature, while the H and N contents decrease, which may indicate more unsaturated character.

The ^{13}C CP-MAS spectra (Figure 2) show in more details the similarities and differences in the chemical structures between the melanoidins Am, Bm and Cm. The aliphatic region of the melanoidins Bm and Cm are similar. Both show two major resonances at ≈ 70 and ≈ 32 ppm but with reversed intensities. The resonance at 70 ppm, attributed to a tertiary C bearing either an O or N atom, is more intense in Bm than in Cm, while the 32-ppm resonance (secondary C linked to an O or N atom, quaternary C linked to N) is less in Bm than in Cm. The aliphatic region of Am, while also having a resonance at 70 ppm, does not show a major signal at 32 ppm but instead shows one at 46 ppm (secondary C linked to O and/or a tertiary C linked to a C atom). All three melanoidins show a resonance at 13 ppm, which very likely is a methyl C. In the unsaturated region, both Bm and Cm show a major resonance at 110 ppm. This resonance usually arises from the presence of either alkene or aromatic moieties. In contrast, in the spectrum of Am, there is a relatively small broad signal centered at ≈ 105 ppm. The broad signals at 132 ppm (aromatic, heteroaromatic C) and 152

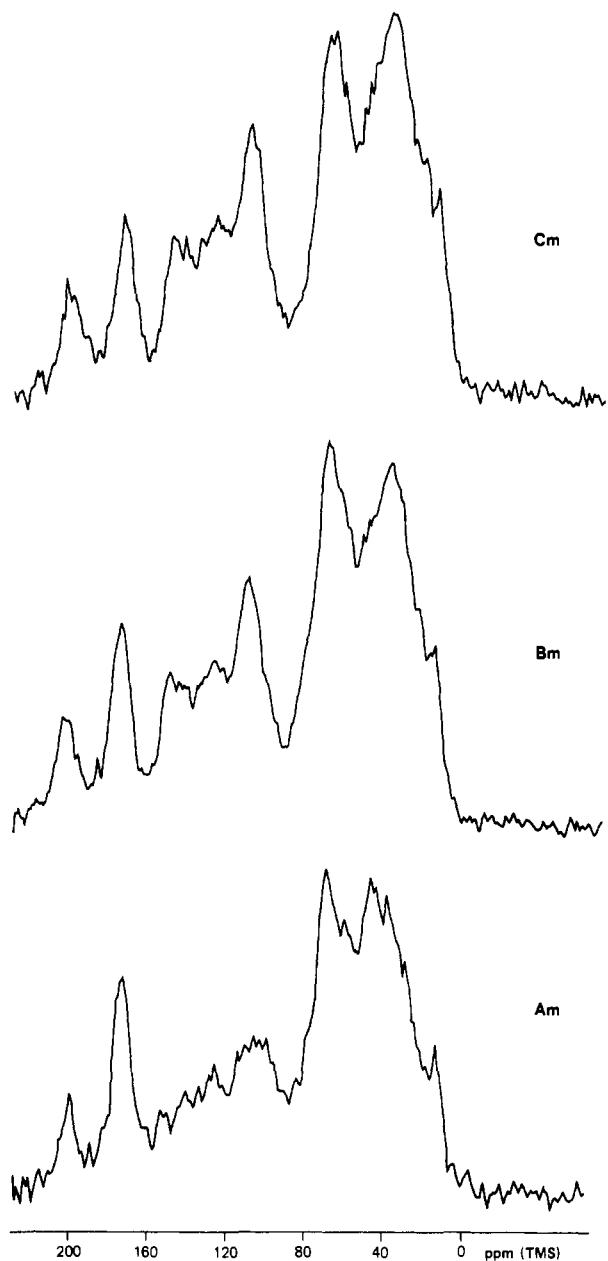


Figure 2. ^{13}C CP-MAS spectra of melanoidins Am, Bm, and Cm synthesized at 22, 68, and 100 $^{\circ}\text{C}$.

ppm (C bearing an O or N in an aromatic ring or C α to N), in Bm and Cm are almost nonexistent in Am. In the carbonyl region (160–220 ppm), the major difference lies in the intensities of the resonance ≈ 200 ppm, the resonance in Am being less prominent.

In conclusion, the ^{13}C CP-MAS spectra show that an increase in reaction temperature causes a major increase in aromaticity in the melanoidins with only a slight decrease in the carbonyl character. These data not only corroborate the microanalytical data but also are in line with the IR data. The major differences in the IR spectra are in the intensities of the two bands at 1710 and 1550 cm^{-1} . Both bands increase in intensities with temperature. In Am, the bands are only shoulders, and the 1710- cm^{-1} band is shifted toward 1730 cm^{-1} . The above results are also in agreement with the data on melanoidins synthesized from glucose and glycine at temperatures at 70, 80, and 90 $^{\circ}\text{C}$ (Bobbio et al., 1981). From the IR and microanalytical data, these authors concluded that these molecules were highly unsaturated. Examination by ^{13}C NMR of the soluble fractions, Bs and Cs showed the same trend (Figure

1). With the exception of the two resonances at 172 and 41 ppm, due to glycine, and the small resonances due to residual amounts of xylose (Figure 1), the soluble fraction Bs shows only 12 carbon resonances that are due to a single compound: the Amadori product resulting from the reaction of two xylose molecules with the amino group of glycine (Benzing-Purdie and Nikiforuk, 1984). A similar 12-carbon product was one of two compounds previously isolated from this reaction by Anet (1959). By contrast, the ^{13}C spectrum of Cs is much more complicated and shows, like the melanoidin isolated from the mixture, much more aromatic character. There is a group of peaks in the 128–136-ppm region, corresponding to either alkene, aromatic, or heteroaromatic carbons, which could be attributed to phenols, pyrroles, pyridines, etc. Such compounds have been isolated from reactions of xylose and glycine (Miller et al., 1981). In the ^{13}C spectrum of Cs, there is a peak at 157 ppm, which may be due to an azomethine moiety, ester, or anhydride, and also more peaks at 170 ppm attributable to acids, esters, or amides. In addition, this soluble fraction shows the presence of the same 12-carbon compound found in Bs but in smaller quantity.

The general conclusion that can be reached from these studies is that, in the Maillard reaction, where equimolar amounts of starting materials are used, an increase in temperature leads to an increase in aromatic character in both high and low molecular products. These studies also show that the structure of the melanoidin synthesized at room temperature (22 $^{\circ}\text{C}$) differs considerably from those synthesized at higher temperatures in that it has different types of aliphatic carbons and fewer unsaturated carbons.

ACKNOWLEDGMENT

We thank P. Benoit and J. Nikiforuk for technical assistance and H. Seguin of the National Research Council of Canada, Ottawa, for the microanalytical data.

Registry No. D-Xylose, 58-86-6; glycine, 56-40-6.

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Received for review January 30, 1984. Revised manuscript received August 10, 1984. Accepted October 9, 1984. C.B.R.I. Contribution No. 1444. Presented in part at the 186th National Meeting of the American Chemical Society, Washington, DC, Aug 28–Sept 2, 1983, AGFD 8.