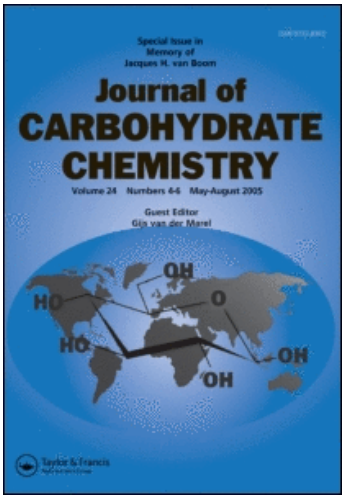

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Solar State ^{1}H and ^{13}C NMR Studies of Melanoidins Synthesized from D-Xylose and Glycine

L. M. Benzing-purdie[a]; C. I. Ratcliffe[b]; J. A. Ripmeester[b]
[a] Plant Research Centre, Agriculture Canada, Ottawa, Ontario [b] Division of Chemistry, National Research Council, Ottawa, Ontario, Canada

To cite this Article Benzing-purdie, L. M. , Ratcliffe, C. I. and Ripmeester, J. A.(1986) 'Solar State ^{1}H and ^{13}C NMR Studies of Melanoidins Synthesized from D-Xylose and Glycine', Journal of Carbohydrate Chemistry, 5: 4, 571 — 584
To link to this Article: DOI: 10.1080/07328308608062976
URL: http://dx.doi.org/10.1080/07328308608062976

J. CARBOHYDRATE CHEMISTRY, 5(4), 571-584 (1986)

SOLID STATE ^{1}H AND ^{13}C NMR STUDIES OF MELANOIDINS SYNTHESIZED FROM D-XYLOSE AND GLYCINE

L.M. Benzing-Purdie*[1], C.I. Ratcliffe[2] and J.A. Ripmeester[2]

1.Plant Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6

2.Division of Chemistry, National Research Council, Ottawa, Ontario, Canada K1A 0R6

Received May 9, 1986 - Final Form August 8, 1986

ABSTRACT

Parameters affecting ^{13}C CP/MAS NMR spectra of solid melanoidins (in particular ^{1}H T_1, $T_{1\rho}$ and the cross polarization time t_{cp}) have been obtained with the aim of determining the optimum conditions necessary for quantitative studies. Melanoidins were synthesized at 22°, 68° and 100 °C, from molar solutions of D-xylose and glycine. The ^{1}H $T_{1\rho}$ values for all melanoidins were similar. Single exponential ^{1}H T_1 and ^{1}H $T_{1\rho}$ relaxation curves were observed for all three polymers, suggesting that the materials were relatively homogenenous. An increase in T_1's with an increase in unsaturation was also observed. The optimum conditions for quantifying the different types of C were found to be t_{cp} = 2 msec., recycling time 2 sec. and for these conditions, the melanoidins synthesized at 22°C showed 18% unsaturation, 18% carboxyl and amide C, while the melanoidin synthesized at 100 °C showed 28 and 11% respectively. Solid state ^{1}H NMR lineshapes were obtained and these consisted in all three melanoidins of a broad and a narrow component attributed to the protons of the polymer core and the protons of the mobile side chains or methyl groups respectively.

0732-8303/86/0504-0571$3.50/0

INTRODUCTION

Melanoidins, the brown nitrogenous polymers formed in the reaction of reducing sugars and amino compounds, originally described by Maillard in 1912[1], have been the subject of many studies. These high molecular weight compounds first compared to humic substances[2] by Maillard, are also important components of our daily diet. Even though several symposia[3,4,5], have in part dealt with their properties, there is still a large gap in our knowledge concerning the chemical structure, partly because of the complexity of the reaction itself. Temperature, time, moisture content, concentration and nature of reactants are all important factors in this amino-carbonyl reaction.

Recently it was found by ^{13}C CP-MAS NMR, that melanoidins synthesized from D-xylose and glycine showed with time[6] and temperature[7], an increase in unsaturated character and decrease in total C=O content. Microanalysis showed at the same time, an increase in C and a decrease in N content. Although the conclusions of the latter work remain valid, no attempt has so far been made to quantify the different types of carbons.

In view of the recently found correlation between the biodegradability of these polymers by soil microorganisms and their unsaturated and total carboxyl, amide ester and carbonyl content[8], we have explored the possibility of using solid state ^{13}C NMR as a rapid method for quantifying these carbons.

RESULTS AND DISCUSSION

^{1}H NMR spectra

The proton NMR spectra (Fig. 1) of the three melanoidins synthesized at different temperatures, consist of two components,

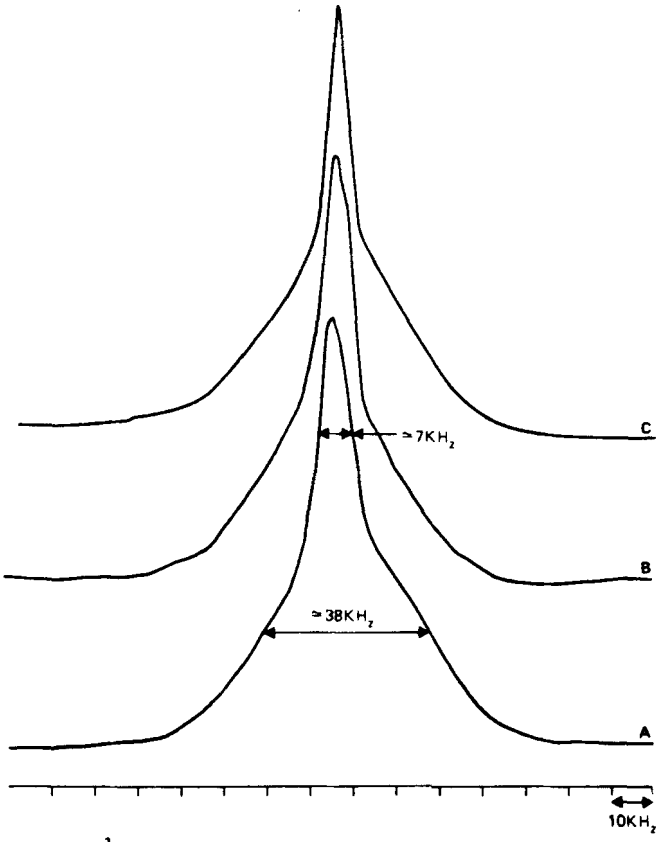

Fig. 1 ^{1}H NMR spectra of melanoidins A, B and C

one broad and one narrow component with width at half height of ≃38 and 6-7 kHz respectively. These widths are governed by proton dipole-dipole interactions. Considering the general nature of the melanoidin polymers, i.e. the presence of methyl, methylene, methine, and unsaturated group protons, some conclusions can be made without doing a detailed analysis. The broad and narrow components reflect differing degrees of motional averaging, with the broad component representing protons in the core polymer and the narrow component representing protons in mobile side chains and methyl groups.

TABLE 1. ^{1}H relaxation parameters

Melanoidins	T_1 (msec)	$T_{1\rho}$ (msec)
A	2.80	39.8
B	3.62	194
C	4.43	207

The ^{1}H relaxation parameters (Table 1) show that ^{1}H $T_{1\rho}$ values for the three melanoidins fall within the usual range of ^{1}H $T_{1\rho}$ for polymers[9]. They do not differ greatly from one another; the temperature of synthesis being not very critical. Unlike ^{1}H $T_{1\rho}$, the ^{1}H T_1's, however, vary. The observation of single exponential relaxations for all three polymers, suggests that the materials are relatively homogeneous, so that spin diffusion to mobile groups and radical centres takes place efficiently even on the short $T_{1\rho}$ time scale of several msec.

The melanoidins C and B synthesized at 100° and 68 °C have similar ^{1}H T_1's, while that for melanoidin A synthesized at 22 °C is much shorter. Although paramagnetic impurities considerably shorten ^{1}H T_1's[10], in this particular case the small T_1 value is not due to that effect. ESR measurements[11] showed very little difference between the three melanoidins.

The trend of increased relaxation time with increased unsaturation of the polymer as observed with an increase in reaction temperature, parallels the trend observed in low to medium rank coals[12,13], i.e. the ^{1}H T_1 increases with increasing coal rank. This parallel trend may reflect the increase in polymer rigidity as the unsaturated or aromatic character of the material increases. Since the ^{1}H lineshapes are very similar, the ^{1}H-^{1}H dipolar interactions are averaged to the same degree, i.e. the amplitudes of molecular motion in the polymers are the same. The difference in relaxation times must therefore reflect a different motional rate, i.e. a slower motional rate with increase in relaxation time.

^{13}C NMR spectra

Previously reported spectra[7] of melanoidins synthesized at 22°C, 68° and 100°C were recorded at 45.28 MHz at a spinning rate of 3.4 kHz. Under the latter conditions, the spinning side bands of the aromatic and carbonyl carbon resonances contribute to the intensities of the signals situated at ~75 ppm on either side of the main peaks to the extent of 10% for carbonyl carbons up to 25% for aromatic carbons. In order to minimize these interferences, for quantitative measurements, the spectra were recorded at the same frequency but at a higher spinning rate (5.3 kHz). In this case, spinning side bands occur at 115 ppm on either side of the main peaks, and have no more than 5% of the intensity of the central bands. Contribution of the spinning side bands of aromatic peaks to the spectrum, may be found in the 0-30 ppm region.

The spectral features of each melanoidin can be readily distinguished in Fig. 2. The spectra can be arbitrarily divided into the five regions: 0-60 ppm (alkyl C), 60-110 ppm (C bonded to O or N), 110-160 ppm (unsaturated C), 160-190 ppm (carboxyl, amide or ester C), 190-220 ppm (carbonyl C). Overlap of different kinds of C can occur, e.g. resonances for both unsaturated and acetal C are found at 110 ppm. While the spectra of the melanoidins synthesized at 100° and 68°C show the same signals, the spectrum of the melanoidin synthesized at 22°C, does not display the well defined signals in the 120-150 ppm region attributed to aromatic or heteroaromatic C.

In a CP-MAS NMR experiment, the proton magnetization is spin locked with a proton radio frequency field. During this period, the sample is irradiated at the ^{13}C frequency for a period referred to as the contact time (t_{cp}), during which there is a transfer of magnetization from the proton spin system to the carbon spin system, provided the Hartmann/Hahn cross-polarization conditions are met[14]. The ^{13}C magnetization grows according to the rate of polarization transfer, but at the same time there is a competing decay according to the proton spin lattice relaxation time in the rotating frame ^{1}H $T_{1\rho}$. The proton magnetization is reestablished

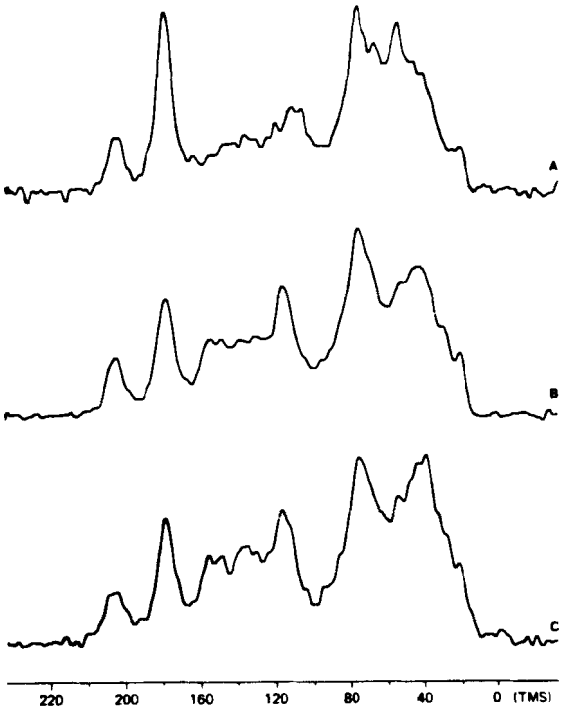

Fig. 2 CP-MAS ^{13}C NMR spectra of melanoidins A, B and C (cross polarization time of 1 msec.)

during the delay time. This latter process is characterized by the ^{1}H spin lattice relaxation time $^{1}H\ T_1$. While the $^{1}H\ T_1$ values are the same for all types of protons because of rapid spin diffusion, the rates of polarization transfer from ^{1}H to ^{13}C must vary from one chemically distinct type of carbon to another dependent on the strength of its interactions with neighbouring protons. Consequently, some ^{13}C signals reach maximum intensity at shorter t_{cp} times than others. One must therefore determine which values of t_{cp} are sufficiently long to give quantitative ^{13}C spectra.

Fig. 3 and 4 are illustrations of the effect of contact time (t_{cp}) on the spectra of melanoidins A and C, synthesized at low and high temperature. The signal intensities are clearly very sensitive to contact time. For melanoidin A, maximum signal

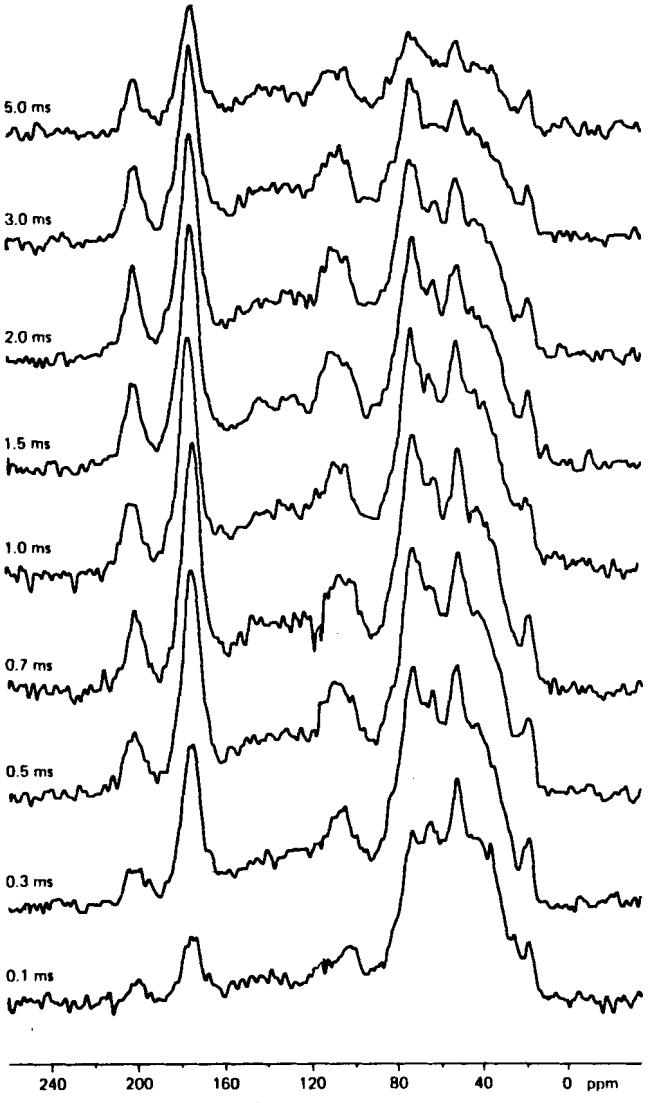

Fig. 3. CP-MAS ^{13}C NMR spectra of melanoidin A as a function of cross polarization time (t_{cp}).

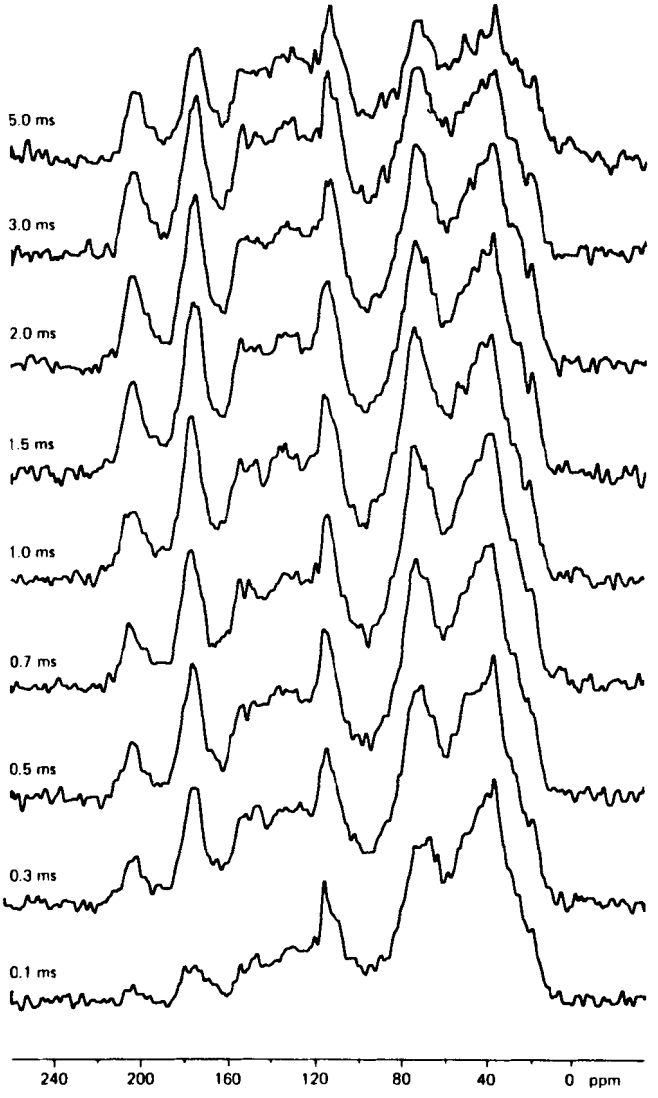

Fig. 4. CP-MAS ^{13}C NMR spectra of melanoidin C as a function of cross polarization time (t_{cp}).

TABLE 2. Intensities of the signals for melanoidin A[15].

Contact time (msec)	Chemical Shift Range (ppm)				
	0-60	60-110	110-160	160-190	190-220
0.1	76	67	20	13	4
0.3	73	93	36	31	7
0.5	87	98	49	44	13
0.7	82	102	51	47	13
1.0	82	100	51	47	13
1.5	84	89	49	47	13
2.0	71	73	44	44	13
3.0	58	64	38	38	11
5.0	36	40	29	24	7

intensities for all C were achieved at $t_{cp} \simeq 0.7$ msec. (Table 2), while maximum signal intensities in melanoidin C (Table 3) were achieved at $t_{cp} \simeq 0.7$ msec. for alkyl C (0-60 ppm) and C bonded to O and N (60-110 ppm) and a $t_{cp} \simeq 1.5$ msec for unsaturated C (110-160 ppm), carboxyl, amide or ester C (160-190 ppm) and carbonyl C (190-220 ppm). At $t_{cp} = 5$ msec., all the intensities had declined in melanoidin A, e.g. 40-60% of the intensities of the signals were lost.

Figure 5 shows the relative intensities (expressed as a percentage of the integrated intensity of the whole spectrum) of the unsaturated C (110-160 ppm) and the carboxyl, amide, ester C (160-190 ppm) for the two melanoidins versus t_{cp}. In the case of the melanoidin A synthesized at low temperature, at the 1 msec cross polarization time used in earlier experiments, the C=C and the COO,CONH carbons are only slightly underestimated as 17 and 17.5% versus 18 and 18%, respectively, at $t_{cp} = 3$ msec. In melanoidin C synthesized at higher temperature, however, that underestimation is more pronounced, in particular for the C=C carbons, 25% ($t_{cp} = 1$ msec) versus 29% at $t_{cp} = 3$ msec.

Following each pulse sequence, sufficient time must be allowed

TABLE 3. Intensities of the signals for melanoidin C[15].

Contact time (msec)	Chemical shift range (ppm)				
	0-60	60-110	110-160	160-190	190-220
0.1	89	65	41	8	3
0.3	103	86	73	24	8
0.5	114	92	78	27	11
0.7	119	92	81	30	14
1.0	116	100	92	35	16
1.5	108	97	100	41	19
2.0	103	95	97	38	19
3.0	86	81	89	35	16
5.0	76	62	73	27	11

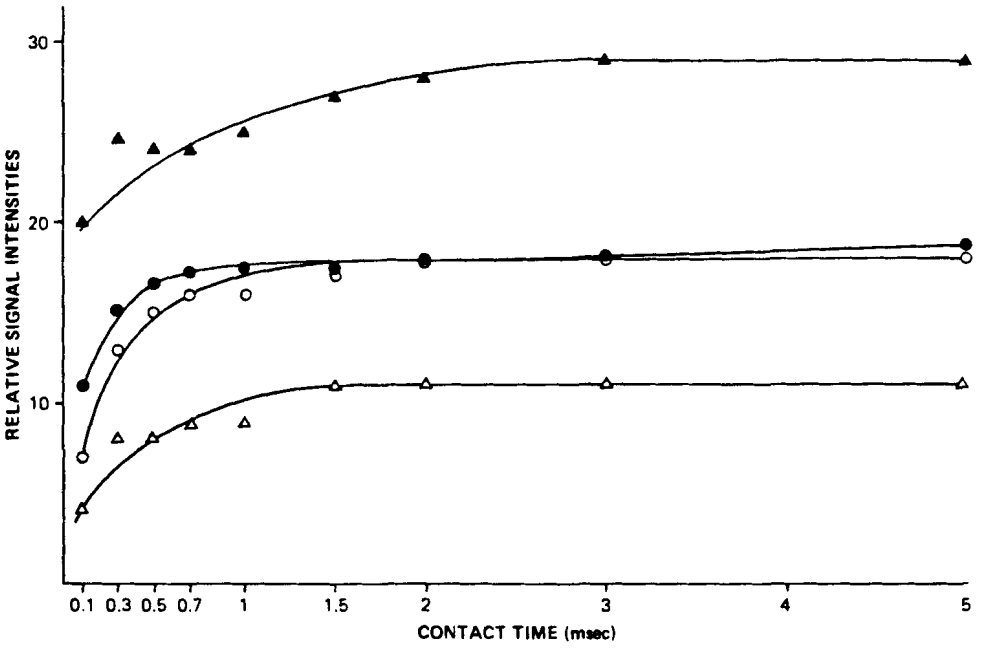

Fig. 5 Relative intensities of the C=C (110-160 ppm) and COO,CONH (160-190 ppm) carbons in melanoidins A (△-△, ○ - ○) and C (▲-▲, ●-●).

for the ^{1}H magnetization to return to its equilibrium state. At 5 times T_1, this recovery is more than 99% complete. It is therefore clear from the T_1 values in Table 1, that a recycle time of 2 seconds is more than adequate for the three melanoidins studied here.

CONCLUSION

The data show that it is possible to use ^{13}C CP-MAS NMR as a means of quantifying both unsaturated and carboxyl, amide, or ester carbons in melanoidins. Although the discussion above centers around these carbons, this same analysis may be applied to other carbons, e.g. alkyl. A contact time of 2 msec, and a recycle time of 2 sec. appears to be a good compromise for quantitative results, if one is dealing with a variety of melanoidins. Under the latter conditions, the melanoidins synthesized at 100 ^{o}C showed 28% unsaturation with 11% carboxyl, amide or ester carbons, while the melanoidin synthesized at 22 ^{o}C showed 18% for both types of carbons.

EXPERIMENTAL

^{1}H NMR

^{1}H NMR lineshapes were obtained at 60 MHz by use of a Bruker 1.413T electromagnet and a Bruker CXP 180 spectrometer. A "magic echo" pulse sequence was used as described[16] and the spectra were obtained by Fourier transformation of the signal beginning at the peak of the echo.

^{1}H spin lattice relaxation times (T_1) and ^{1}H rotating frame spin lattice relaxation times ($T_{1\rho}$).

^{1}H T_1 and T_1 measurements were obtained using a Bruker SXP spectrometer operating at 60 MHz. A 180°-t-90° pulse sequence was used to measure T_1. T_{1p} was measured by means of a 90° (X) pulse followed by a 90° phase shifted (Y) pulse of variable duration. The 90° pulse lengths were 5 μsec., corresponding to a spin-locking field H_1 of 11.74G for the $T_{1\rho}$ experiment. The digitized free induction decays were collected in a Nicolet LAS 12/70 data processor and then transferred to a PDP RT 11 VO4 computer where linear least squares fits of ln $(M_o-M_t/2M_o)$ vs t were carried out to obtain T_1 or $T_{1\rho}$.

^{13}C CP-MAS NMR

The spectra were recorded by means of a Bruker CXP 180 spectrometer at a frequency of 45.28 MHz. Experimental details were similar to those described earlier[17], except that spinning rates of the order of 5.3 kHz were obtained using a probe purchased from Doty Scientific Inc. Cross polarization times varying from 0.1 to 5 msec. and a recycle time of 2 sec. were used. Signal intensities were obtained by integration.

Synthesis of melanoidins

Melanoidins A, B and C were synthesized at 22°, 68° and 100 °C from molar solutions of D-xylose and glycine under sterile conditions, as previously reported[7].

ACKNOWLEDGEMENT

The authors wish to thank J. Nikiforuk for technical assistance.

REFERENCES AND FOOTNOTES

1. L.C. Maillard, Compt. Rend. Acad. des Sciences, 154, 66 (1912).

2. L.C. Maillard, Compt. Rend. Acad. des Sciences, 156, 1159 (1919).

3. C. Ericksson, Ed.; "Progress in Food and Nutritional Science - Maillard Reactions in Food". Pergamon Press, New York, N.Y. Vol. 5, 1982.

4. G.R. Waller and M.S. Feather, Eds.; "The Maillard Reaction in Foods and Nutrition", American Chemical Society, Washington, D.C., ACS Symposium Series No. 215, 1983.

5. M. Fujimaki, M. Namiki and H. Kato, Eds.; Developments in Food Science Vol. 13, "Amino-carbonyl Reaction in Food and Biological Systems". Elsevier, Tokyo, Japan, 1986.

6. L.M. Benzing-Purdie and C.I. Ratcliffe in; "Developments in Food Science" Vol. 13. Amino-carbonyl Reaction in Food and Biological Systems. M. Fujimaki, M. Namiki and H. Kato, Eds.; Elsevier, Tokyo, Japan, 1986, p. 193.

7. L.M. Benzing-Purdie, J.A. Ripmeester, C.I. Ratcliffe, J. Agric. Food Chem., 33, 31 (1985).

8. L.M. Benzing-Purdie and K.C. Ivarson, Abstract Papers, Third International Symposium on the Maillard reaction, Fuji Institute, Japan, July 1-5, 1985. B-76, p. 90.

9. L.B. Alemany, D.M. Grant, R.J. Pugmire, T.D. Alger and K.W. Zilm, J. Am. Chem. Soc., 105, 2133 (1983).

10. J. Haupt and W. Muller-Warmuth, Z. Naturforsch. 229, 643 (1967).

11. B.A. Goodman, Macaulay Institute for Soil Research, Aberdeen, Scotland, U.K. Personal communications.

12. T. Yokono and Y. Sanada, Fuel, 57, 334 (1978).

13. B.C. Gerstein, C. Chow, R.G. Pembleton and R.C. Wilson, J. Phys. Chem., 81, 565 (1977).

14. A. Pines, M.C. Gibby and J.S. Waugh, J. Chem. Phys. 59, 569 (1973).

15. The value at t_{cp} = 1 msec for the range 60-110 ppm was arbitrarily given the value 100 to effect a normalization.

16. P.L. Kuhns, M.S. Conradi, J. Chem. Phys., 80, 5851 (1984).

17. L.M. Benzing-Purdie and J.A. Ripmeester, Soil Sci. Soc. Am. J., 47, 56 (1983).

18. Plant Research Centre contribution No. 1005