Transformation of Niacin-Containing Compounds in Corn during Grain Development: Relationship to Niacin Nutritional Availability[1]

Joseph S. Wall,* Michael R. Young, and Kenneth J. Carpenter

Most niacin in mature field corn is nutritionally unavailable to rats; however, niacin derivatives in immature sweet corn promote growth of rats and yield nicotinamide upon boiling. To investigate these differences, meals and extracts of field and sweet corn harvested at milky, dough, denting, and mature stages of grain development were analyzed for total niacin and niacin-containing compounds. Nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), nicotinic acid, nicotinamide, trigonelline, and other substances were determined in the extracts by thin-layer and high-performance liquid chromatography. In both field and sweet corn in the milky stage, NAD and NADP are the major niacin-containing substances. As grain matures, some nicotinamide and nicotinic acid are formed and, finally, bound niacin and trigonelline predominate. The presence of nicotinic acid primarily in NAD and NADP in milky sweet and field corn appears related to the biological availability of niacin in these grains.

Human diets based largely on corn meal have been associated with pellagra, a niacin (nicotinic acid) deficiency disease (Carpenter, 1981). Analysis of alkali-hydrolyzed mature field corn grain samples established the presence of moderate amounts of the vitamin in the grain, 27.3 μg as nicotinic acid/g of corn; of this, only 7.0 μg/g was biologically available to rats as indicated by their growth (Carter and Carpenter, 1982). Kodicek (1940) attributed the inability of most of the corn niacin to support animal growth to its being bound in a chemical linkage that is not disrupted during assimilation. Kodicek et al. (1956), Pearson et al. (1957), and Harper et al. (1958) established that the niacin liberated when corn was heated in the presence of dilute alkali was biologically available. Christianson et al. (1968) suggested that bound nicotinic acid in corn was combined with carbohydrate by an ester linkage. Mason et al. (1973) and Mason and Kodicek (1973) isolated and characterized a bound nicotinic acid fraction from wheat bran that was similar to the bound nicotinic acid in corn.

It was, therefore, of interest that Carter and Carpenter (1982) reported that most of the niacin (78.0%) in immature sweet corn (harvested at an early stage of development for consumption as a vegetable) *was* available to support growth; colorimetric analysis indicated 51.3 μg of total niacin/g of dry sweet corn, of which 40 μg/g was biologically available, as determined by rat growth. They further observed, by thin-layer chromatography of extracts of the grain, that, after boiling, 45 μg of nicotinamide is released/g of dried immature sweet corn grain. In contrast, the nicotinic acid or nicotinamide-containing compounds in mature field corn are not broken down during boiling.

We sought to establish whether differences in niacin compounds between immature sweet corn and mature field corn were due to differences in genotype (sweet corn contains the *su1* mutant gene) or to differences in stage of maturity (sweet corn is commonly eaten at the immature milky or dough stages of grain development). Also, we investigated the nature of the heat-labile niacin compounds in sweet corn that are biologically available. To explore these problems, both field and sweet corn were harvested at four different stages of grain development: milky, dough, denting, and mature. Extracts of these grains were analyzed for nicotinic acid containing compounds such as nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), nicotinamide mononucleotide (NMN), nicotinic acid adenine dinucleotide (NAAD), nicotinic acid, nicotinamide, and trigonelline (*N*-methylnicotinic acid); biologically unavailable bound niacin was approximated by difference. Colorimetric analysis, thin-layer chromatography (TLC), and reversed-phase and ion-exchange high-performance liquid chromatography (HPLC) were employed to analyze extracts of the grains, or of boiled or alkali-treated grains for these substances.

MATERIALS AND METHODS

Sample Preparations. Golden Harvest 2480 hybrid corn (Sommer Brothers Seeds, Pekin, IL) and Trigold hybrid sweet corn (Sun Seeds, Eden Prairie, MN) seeds were planted in separate fields near Princeville, IL, in 1984, and the ears were harvested at about 21, 28, 35, and 49 days after pollenation to attain milky, dough, denting, and mature stages, respectively, of grain development. The corn was frozen on the cob by packing in dry ice. The immature grain was hand-cut from the cob while frozen, and the frozen mature grain was separated from the cob by a CeCoCo corn sheller. The frozen grain was weighed, lyophilized to dryness, and weighed again. The frozen dry grain was ground in a Udy Cyclone mill to 40 mesh in the presence of dry ice. Moisture and micro-Kjeldahl nitrogen were determined on lyophilized grain according to AOAC (1980) procedures.

Total nicotinic acid in corn samples after hydrolysis with $Ca(OH)_2$ was determined colorimetrically by reaction with cyanogen bromide followed by sulfanilic acid, according to the procedure outlined in AOAC methods 43.044–43.046 (1980). Mature grain samples yielded filtered $Ca(OH)_2$ hydrolysates that were turbid and were clarified by passage through a Millipore CX immersible ultrafilter prior to reaction with cyanogen bromide. All analyses were conducted in duplicate , and results are given as mean values.

Ethanolic extracts of corn meals were prepared by stirring 5 g of ground lyophilized corn with 50 mL of 50%

Northern Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, Peoria, Illinois 61604 (J.S.W., M.R.Y.), and Department of Nutritional Science, University of California, Berkeley, California 94720 (K.J.C.).

[1]Presented at the 69th Annual Meeting of the Federation of Societies for Experimental Biology, April 21–26, 1985, Anaheim, CA.

0021-8561/87/1435-0752$01.50/0

ethanol–water for 30 min. The ethanolic extraction was employed earlier by Kodicek (1940) to extract free and bound niacin without decomposing the latter. The dispersion was centrifuged at 4 °C in a Beckman L8-M ultracentrifuge for 20 min at 15000 rpm, and the supernatant was decanted. The residue was reextracted and centrifuged as above, and the supernatants were combined. This extract was concentrated in a rotary evaporator at 35 °C under vacuum to remove ethanol, and the remainder was lyophilized. The dried solids were dissolved in 10 mL of water and centrifuged in a Beckman L8 M ultracentrifuge at 20000 rpm. For TLC, 5 mL of extract was shaken with 3 mL of trichloroethylene and centrifuged; the supernatant was concentrated to dryness and taken up in 2 mL of water (Carter and Carpenter, 1981). For HPLC, the extract supernatant from ultracentrifugation was filtered through a 0.45-μm membrane filter. To study the effect of alkali treatment on the extracts used for TLC, 1 mL of each ethylene chloride treated concentrated extract was mixed with 1.0 mL of 2 M NaOH, heated at 100 °C for 1 h, and neutralized with 1 mL of 2 M HCl.

The effect of boiling corn was demonstrated by boiling the ground meals in 5 volumes of water for 30 min. This time was chosen since it represents common cooking practice for sweet or field corn. The entire cooked dispersion was lyophilized and extracted with 50% ethanol as above.

We also extracted NAD, NADP, and other nucleotides from corn meal by the procedure of Wynants and Van Belle (1985), with modification for seed tissues since this procedure optimizes extraction of the nucleotides. Lyophilized ground corn meal (4.0 g) was twice homogenized with a Brinkman Polytron homogenizer in 20 mL of ice-cold 0.6 N $HClO_4$ for 15 s; the extract was centrifuged at 4 °C at 20000 rpm in the Beckman L8 M ultracentrifuge. To 600 μL of supernatant was added 400 μL of ice-cold 1 M $NaHCO_3$ for neutralization. This solution was recentrifuged in a Beckman TL-100 centrifuge. From 20 to 60 μL of supernatant was injected for reversed-phase HPLC.

Chromatographic Separations. Thin-layer chromatography was conducted as described by Carter and Carpenter (1981) on plates precoated with silica gel 60 F-254 (E. Merck, Darmstadt, Germany), which was activated by heating at 160 °C for 1 h. The equilibrated plates on which samples were applied were developed with water-saturated 1-butanol, dried, exposed to CNBr vapor for 1 h, and sprayed with 2% 4-aminobenzoic acid in 0.75 M HCl–95% ethanol (3:1).

HPLC was used to resolve compounds in extracts and to establish identities of separated compounds. Analyses were performed on a Spectro Physics SP 8700 ternary gradient system with an A 1300-10 dynamic mixer. Samples were applied to columns with a Waters WISP 710B automatic sample injector. Compounds were detected in column effluents with a Beckman 165 variable dual-wavelength UV analyzer at 254 nm and 280 or 340 nm at 0.1 absorbance unit (AU) full scale. Data were recorded on a Kipp and Zonen BD 41 recorder and also stored, integrated, and replotted by means of a Modcomp Model 4 computer.

Compounds used for standards were the purest available from Sigma. Methanol and 1-butanol for chromatography were HPLC grade from Burdick and Jackson. All buffers and reagent chemicals were Fisher C. P. grade. Water was deionized on a Barnsted Nanopure system to 15 MΩ cm.

The compounds in both 50% ethanol and 0.6 M perchloric acid extracts of corn were best separated by reversed-phase HPLC on a Beckman C_{18} 5-μm Ultrasphere ODS 4.6 × 250 mm column with a flow rate of 1 mL/min at room temperature. The elution system was similar to that of Payne and Ames (1982). Solvent A was 0.25 M ammonium acetate, pH 6; solvent B was 40% methanol–60% solvent A. The series of steps and linear gradients used was as follows: 0–5.0 min, 100% A; 5.0–12.5 min, linear gradient to 93.7% A + 6.3% B; 12.5–22.5 min, linear gradient to 75% A + 25% B; 22.5–35.0 min, linear gradient to 100% B. Initial column conditions were restored by a linear gradient from 35 to 45 min to 100% A, and the column was equilibrated at 100% A from 45 to 55 min prior to the next analysis.

For some preliminary experiments, reversed-phase HPLC separations were carried out on Waters μBondapak C_{18} 10-μm columns by using a procedure like that of Miksic and Brown (1977). Flow rate was 1 mL/min at room temperature. Eluents: solvent A, 0.02 M KH_2PO_4, pH 7.0; solvent B, 50% methanol. Steps and gradients: 0–10 min, 100% A; 10–20 min, linear gradient to 90% A + 10% B; 20–40 min, linear gradient to 50% A + 50% B; 40–50 min, linear gradient to 100% B. The column was restored to 100% A by a linear gradient from 50 to 60 min and equilibrated at 100% A from 60 to 70 min.

To determine trigonelline, nicotinic acid, and some other polar compounds, which are poorly resolved by reversed-phase HPLC, separations on a Beckman Spherogel cation-exchange HPLC column (4.6 × 250 mm) were conducted in a manner similar to the macrocolumn procedure of Christianson et al. (1966). Separation was attained by stepwise elution first with 0.2 N pH 3.25 sodium acetate buffer for 30 min, then with 0.2 N pH 4.25 buffer for 20 min, and finally with 0.35 N pH 5.25 buffer for 100 min at 0.7 mL/min flow rate at 50 °C.

To facilitate further identification and quantitation of pyridine nucleotides, they were reduced, elution positions were determined by reversed-phase HPLC, and absorbance at 340 nm was measured. For this purpose 1 mL of ethanolic grain extract (5 g/10 mL) or standard solution of NAD or NADP (40 μg/mL) was treated with 4 mg of sodium dithionite and 10 mg of sodium bicarbonate in a boiling water bath for 90 s and cooled in an ice bath (Beigenhertz et al., 1955).

RESULTS

Corn Grain. Table I summarizes compositional changes during development of field and sweet corn grain harvested at different intervals after pollenation. Both types of grain had high moisture contents at the early stages of grain development, but the lyophilized grain at these early stages had higher protein and total nicotinic acid contents than mature grain, which has a higher carbohydrate content. Similar results were obtained in a series of corn samples grown in 1983 from the same hybrids. Since fewer data were acquired from those samples and variations occurred in grain development due to climate, those data are now detailed.

Comparison of Compositions of Field Corn and Sweet Corn Grain Extracts. Thin-layer chromatography of the concentrated defatted 50% ethanol extracts of both sweet corn and field corn harvested at maturity showed only traces of free nicotinic acid and no free nicotinamide (Figure 1). Most of the cyanogen bromide reacting material in these extracts remained at the origin and probably is bound nicotinic acid. After the corn was boiled prior to extraction, there was no apparent change in the chromatographic pattern. In contrast, the milky stages of both sweet corn and field corn yielded 50% ethanol extracts that showed no bound niacin at the origin (Figure 1). The extract of the milky sweet corn showed

Table I. Moisture, Nitrogen, and Nicotinic Acid Contents of Field and Sweet Corn Grain Harvested at Different Stages of Grain Development

corn type	no. harvest days after pollenation	at harvest, % moisture	after lyophilization % moisture	after lyophilization % nitrogen	after lyophilization nicotinic acid,[a] μg/g
sweet					
milky	21	83.7	6.02	2.50	62.4 ± 2.4
dough	28	65.0	4.49	1.80	44.4 ± 2.2
denting	35	60.6	14.44	1.50	42.0 ± 1.5
mature	49	11.1	9.30	1.94	40.0 ± 2.0
field					
milky	21	77.6	4.95	1.95	52.8 ± 2.2
dough	28	66.4	9.86	1.74	38.3 ± 1.5
denting	35	57.8	8.54	1.55	34.0 ± 1.8
mature	49	25.5	9.70	1.58	27.7 ± 1.9

[a] Total nicotinic acid was determined by cyanogen bromide–sulfanilic acid colorimetric analysis of $Ca(OH)_2$ hydrolysates of duplicate samples.

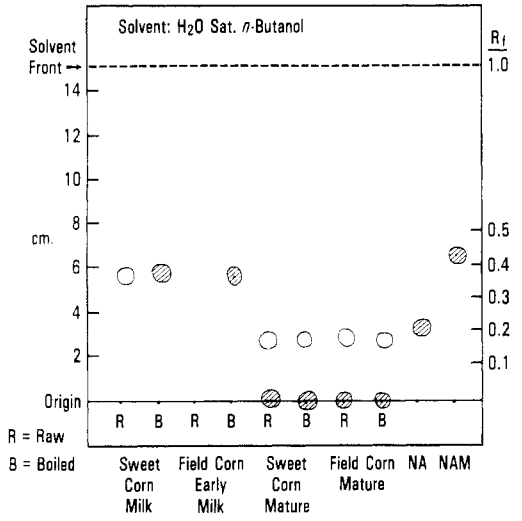

Figure 1. Thin-layer chromatography of 50% ethanol extracts of raw and boiled milky- and mature-stage sweet corn and field corn. Nicotinic acid and its derivatives were revealed by reaction with cyanogen bromide and 4-aminobenzoic acid. Cross-hatched spots indicate intense color; open spots indicate faint color. NA = nicotinic acid, NAM = nicotinamide.

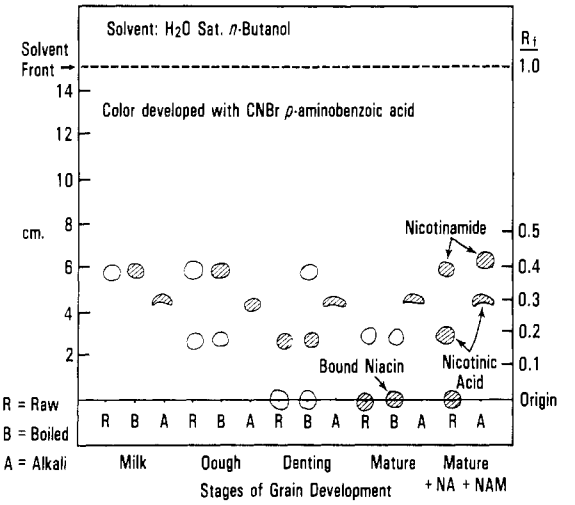

Figure 2. Thin-layer chromatography of raw, boiled, and Ca-$(OH)_2$-treated samples of field corn harvested at different stages of development. Nicotinic acid and its derivatives were revealed by reaction with cyanogen bromide and 4-aminobenzoic acid. Cross-hatched spots indicate intense yellow color; open spots indicate faint color. NA = nicotinic acid, NAM = nicotinamide.

only a trace of nicotinamide, while no cyanogen bromide reacting compounds were observed in the milky field corn extract. After the lyophilized corn meals were boiled in water for 30 min, both the milky sweet corn and milky field corn exhibited pronounced TLC spots (Figure 1) with R_f values identical with that of nicotinamide. These results are consistent with those of Carter and Carpenter (1982) for immature sweet corn and mature field corn. The data indicate that there is no difference in niacin-containing components between field corn and sweet corn at the same stages of development.

Figure 2 illustrates changes in lability of niacin-containing components in field corn grain during development. Standards of nicotinic acid and nicotinamide were co-chromatographed with extracts of the mature grain that were untreated, boiled, and alkali-treated to correct for the effect of salts on mobilities of the two compounds. Boiling releases nicotinamide from heat-labile compounds in milky and dough stages of grain, while $Ca(OH)_2$ hydrolyzes most nicotinic acid containing compounds, including bound nicotinic acid in mature grain, to free nicotinic acid. Thus, a progressive transformation occurs from heat-labile compounds to free nicotinic acid to bound nicotinic acid during grain maturation.

Identity of Heat-Labile Compounds in Milky Corn Grains. We next attempted to identify the heat-labile

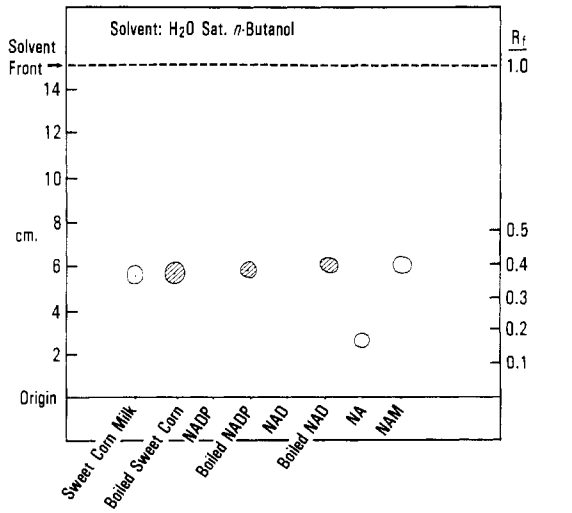

Figure 3. Thin-layer chromatography of boiled NAD and NADP and extracts of raw and boiled milky sweet corn showing nicotinamide formation from heated pyridine nucleotides. Cross-hatched spots indicate intense color; open spots indicate faint color. NA = nicotinic acid, NAM = nicotinamide.

compounds that yield nicotinamide upon boiling of milky sweet and field corn meals. Ethanol (50%) extracts of milky sweet corn meals, boiled and unboiled, were chro-

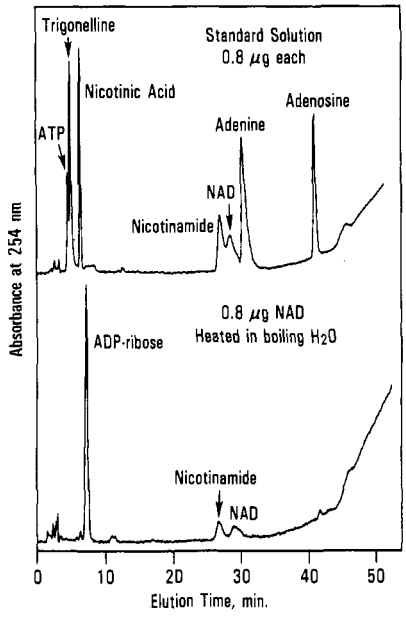

Figure 4. Reversed-phase HPLC on a Waters μBondapak C_{18} column, using the gradient of Miksic and Brown (1977), of degradation products of NAD formed upon boiling.

matographed on thin-layer plates with standards [aqueous solutions (40 μg/mL) of NAD and NADP boiled for 30 min or unboiled] (Figure 3). Heating in aqueous media cleaves the *N*-ribosyl linkage in NAD, NADP, and milky sweet corn constitutents to yield nicotinamide. To verify and quantitate cleavage of NAD and NADP at the *N*-ribosyl linkage, the products of heating were also resolved by reversed-phase HPLC. After NAD was boiled for 30 min, residual NAD (30% of original), nicotinamide (70% of theoretical), and an early eluting peak (presumably ADP-ribose) are present (Figure 4).

The breakdown of NAD and other compounds in boiled corn meals to nicotinamide was next established by reversed-phase HPLC of pyridine nucleotides, nicotinamide, and related compounds in ethanol extracts of boiled and untreated milky sweet corn meals (Figure 5). After the milky sweet corn was boiled for 30 min, there was a marked decrease in NAD, NADP, and NMN peaks and a large increase in nicotinamide.

To further verify that these peaks detected at 254 nm are NAD and NADP, an extract from field corn at milky stage was reduced to dehydro forms with sodium dithionite in the presence of sodium bicarbonate and compared to known reduced pyridine nucleotides by reversed-phase HPLC on a Waters μBondapak C_{18} column (Figure 6) (Miksic and Brown, 1977). Peaks absorbing at 254 and 340 nm, which correspond to elution positions of NADH and NADPH, are present in the corn extract pattern. Heating solutions of NADH and NADPH also gives rise to minor chromatographic peaks indicative of cleavage of the reduced pyridine ring (data not shown).

Quantitation of Nicotinic Acid Derivatives in Corn. Components of extracts of sweet and field corn grain meals at various stages of development were separated by reversed-phase HPLC on Ultrasphere ODS (Payne and Ames, 1982). Examples of separations of 50% ethanol extracts of various stages of field corn (Figure 7) exhibit good resolution of NAAD, NADP, nicotinamide, and NAD from other compounds. A progressive decrease in yields of NAD and NADP with maturity is observed. Amounts of these compounds in corn extracts were calculated from peak areas. For a single extract, duplicate separations gave values for individual compounds that agreed to ±3%.

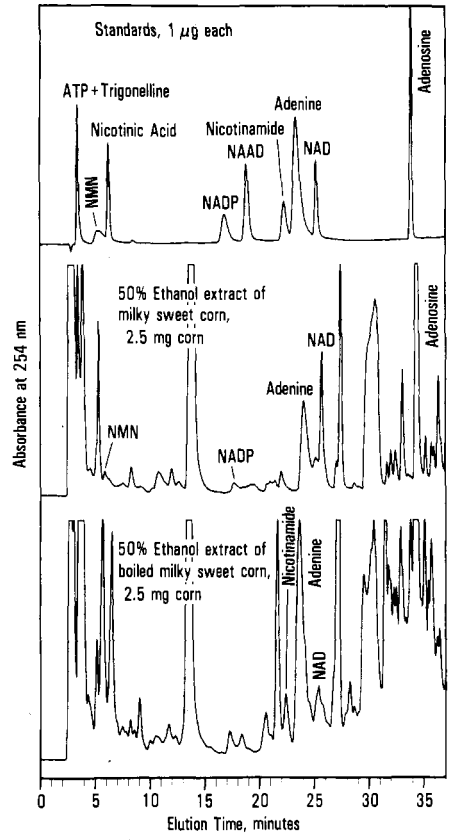

Figure 5. Effect of boiling on degradation of pyridine nucleotides in milky sweet corn. Components in a 50% ethanol extract of boiled and raw sweet corn were separated by reversed-phase HPLC on a Beckman Ultrasphere-ODS column with the gradient system of Payne and Ames (1982).

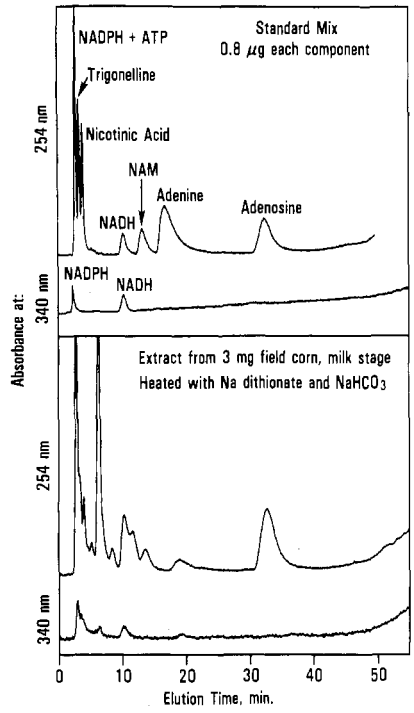

Figure 6. Reversed-phase HPLC of NADH and NADPH in standard mixtures and in dithionite-reduced 50% ethanol extracts of milk-stage field corn on a Waters μBondapak C_{18} column with a gradient similar to that of Miksic and Brown (1977).

Duplicate extractions gave values that generally agreed to ±5%.

Nicotinic acid and trigonelline elute early upon reversed-phase HPLC (Figure 7) and are not well resolved

Table II. Changes in Nicotinic Acid Derivatives in Sweet Corn during Grain Development

compound	μg compd/g lyophilized corn meal				μg nicotinic acid yielded/g lyophilized meal			
	milky	dough	denting	mature	milky	dough	denting	mature
NAD	233[a]	121	84	27	40	28	14	5
	(268)[b]	(104)	(65)	(20)	(46)	(22)	(11)	(3)
NADP	29	14	36	15	5	2	5	2
	(22)	(8)	(18)	(12)	(4)	(2)	(3)	(2)
NMN	14				5			
	(-)				(0)			
NAAD	4	3			0.8	0.5	-	-
	(0)	(0)			(-)	(-)		
nicotinic acid[c]	0	1	3	2	0	1	3	2
nicotinamide[c]	1.0	2.0	-	-	1.0	2.2	-	-
trigonelline[c]	3	7	14	29				
total nicotinic acid in extr					61[d]	42	28	25
					(65)[e]	(45)	(35)	(30)
total nicotinic acid in grain[f]					62	44	42	40
other niacin compds in grain by diff					10	10	20	31

[a] Ethanol extract by reversed-phase HPLC. [b] Perchloric acid extracts values (in parentheses) determined by reversed-phase HPLC. [c] Ethanol extract by ion-exchange HPLC. [d] Ethanol extract by colorimetric analysis of $Ca(OH)_2$ hydrolysate. [e] Perchloric acid extract by colorimetric analysis of $Ca(OH)_2$ hydrolysate. [f] By colorimetric analysis of $Ca(OH)_2$ hydrolysate.

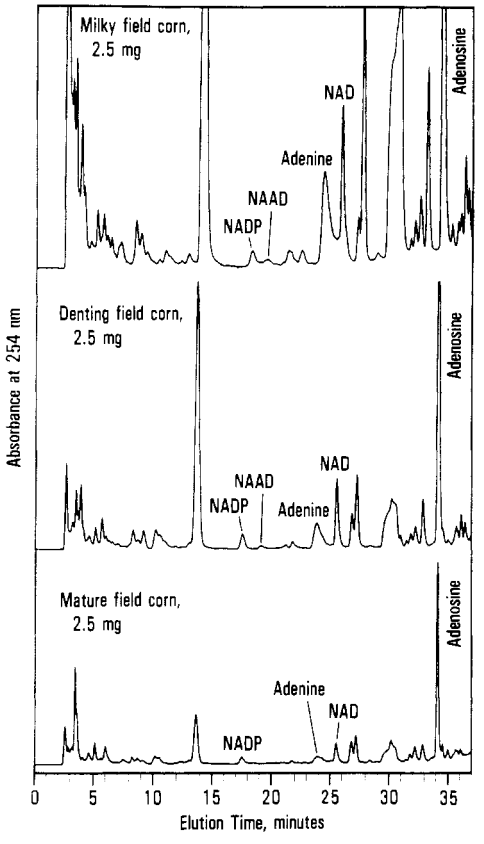

Figure 7. Reversed-phase HPLC of nicotinic acid derivatives and other compounds in 50% ethanol extracts of field corn grain harvested at milky, denting, and mature stages. HPLC was on a Beckman Ultrasphere-ODS column with a gradient similar to that of Payne and Ames (1982).

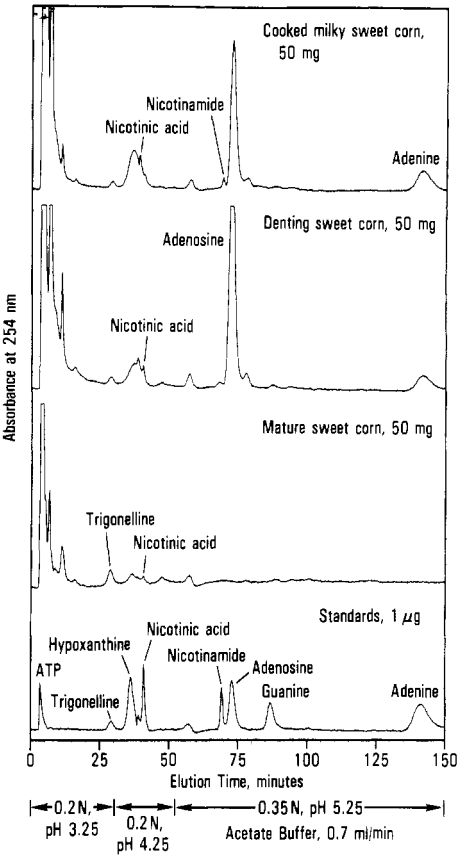

Figure 8. Ion-exchange HPLC of nicotinic acid derivatives in 50% ethanol extracts of boiled milky, denting, and mature sweet corn grain. Separations were performed on a Beckman Spherogel column using stepwise increases in pH and ionic strength.

from other UV-absorbing substances in milky- and dough-stage corn extracts. To better quantitate changes in nicotinic acid and trigonelline in the developing kernel, ethanolic extracts were separated by HPLC on a Beckman Spherogel cation exchange column (Figure 8). Trigonelline, nicotinic acid, and nicotinamide are well retained on the column. The boiled sweet corn extract of the denting stage contains nicotinamide in small amounts, but nicotinamide is absent in mature sweet corn. Nicotinic acid is present in small amounts in both denting and mature grain extracts. Trigonelline progressively increases with maturity.

Table II summarizes calculated yields of the nicotinic acid derivatives extracted with 50% ethanol or 0.6 N perchloric acid from lyophilized meals of sweet corn harvested at various stages of maturity and gives total niacin values determined colorimetrically on alkaline hydrolyzates of grain meal and extracts. Both 50% ethanol and 0.6 N perchloric acid extracted most nicotinic acid derivatives from the milky and dough stage meals; however, the yield of niacin derivatives was incomplete in extracts of mature grain, probably due to poor extraction of bound niacin components. In grain from the milky and dough stages, almost all of the total niacin is NAD and NADP. NMN

Table III. Changes in Nicotinic Acid Derivatives in Field Corn during Grain Development

compound	μg compd/g lyophilized corn meal				μg nicotinic acid yielded/g lyophilized meal			
	milky	dough	denting	mature	milky	dough	denting	mature
NAD	225[a]	119	78	23	39	20	13	4
	(235)[b]	(123)	(63)	(5)	(40)	(21)	(11)	(1.4)
NADP	38	18	32	17	6	3	5	3
	(7)	(–)	(1.0)	(5)	(1)	(–)	(1)	(1)
NMN	–	–	–	–	–	–	–	–
NAAD	8.1	3	3	–	2	0.5	0.6	–
nicotinic acid	–	1	2	1	–	6.0	2	1
nicotinamide[c]	1	2	1	–	1	2	1	–
trigonelline[c]	1	3	7	27				
total niacin extr					51[d]	37	22	24
					(60)[e]	(42)	(33)	(15)
total niacin in grain[f]					53	38	34	28
other niacin compds in grain by diff	1	6			5	12	12	20

[a] Ethanol extract by reversed-phase HPLC. [b] Perchloric acid extract values (in parentheses) determined by reversed-phase HPLC. [c] Ethanol extract by ion-exchange HPLC. [d] Ethanol extract by colorimetric analysis of $Ca(OH)_2$ hydrolysate. [e] Perchloric acid extract by colorimetric analysis of $Ca(OH)_2$ hydrolysate. [f] By colorimetric analysis of $Ca(OH)_2$ hydrolysates.

was present in significant amounts in the 50% ethanol extract of milky sweet corn but was absent in perchloric acid extracts, which had a slightly higher content of NAD. The NMN as well as some of the nicotinamide may be breakdown products of NAD, resulting from the ethanol extraction. The milky sweet corn also contained small amounts of material eluting in the position of nicotinic acid adenine dinucleotide. A small amount of nicotinamide is present in the dough stage, and nicotinic acid is found in the dough, denting, and mature kernel extracts. Trigonelline increases in amount with grain maturity. In mature grain, most nicotinic acid in alkaline meal hydrolysates is not accounted for by the various separated compounds in ethanol or perchloric acid extracts. It is assumed that this nicotinic acid is present as bound niacin.

Table III summarizes quantitative changes in nicotinic acid derivatives in field corn grain harvested at different stages of maturity. At each stage of development, the composition is similar to that of the sweet corn harvested at a similar stage. NAD and NADP account for most niacin in the milky and dough stages. The appearance of nicotinamide in dough-stage meals and nicotinic acid in the denting mature corn presage the formation of bound niacin and trigonelline, the major nicotinic acid derivatives in mature field corn.

DISCUSSION

The difference in niacin biological availability between sweet corn consumed in the immature milky or dough stages and mature field corn (Carter and Carpenter, 1982) is probably not due to genetic differences between hybrids of these two corn genotypes. Analysis of the nicotinic acid derivatives of sweet and field corn at different stages of grain development (milky, dough, denting, mature) established that both types of corn contain similar amounts of specific nicotinic acid derivatives at similar stages of development and that, in both corns, progressive changes in composition occur during grain development. Thin-layer chromatograms of 50% ethanol extracts of field and sweet corn harvested at different stages established that in the milky and dough stages the predominant nicotinic acid containing compounds are heat labile and yield nicotinamide upon boiling. In mature grain most nicotinic acid released by alkali is bound to heat-stable compounds; only a small amount of free nicotinic acid is present.

Several lines of evidence established that heat-labile compounds, predominant in early stages of grain development, are the pyridine nucleotides NAD and NADP. These compounds were shown by TLC and HPLC to be heat labile and to release nicotinamide upon boiling. Reversed-phase HPLC of extracts of corn meals from sweet and field-type hybrids of different degrees of maturity revealed substances with elution times similar to those of known pyridine nucleotides. Furthermore, treatment of corn extracts with sodium dithionite altered elution positions to those of reduced NAD and NADP; characteristic 340-nm absorbance also distinguished these new peaks. Boiling milky corn resulted in reduced levels of pyridine nucleotides in extracts and increased the level of nicotinamide, thereby verifying the lability of compounds with the *N*-ribosyl linkage in boiled grain.

The changes in composition of niacin-containing compounds in corn grain during maturation are consistent with biological needs of the seed. In early stages of grain development the seed is rapidly producing polysaccharides, proteins, and fats for deposition and storage. To supply energy for this activity, the requirements for pyridine nucleotide enzyme cofactors are considerable. As the seed matures, these compounds may be degraded and the formed nicotinic acid mostly transformed into bound niacin for storage. Some pyridine nucleotides are retained in the germ and aleurone to facilitate germination.

The presence of *N*-methylnicotinic acid (trigonelline) in mature corn seed was reported by Christianson et al. (1966). Trigonelline was recently identified as a growth regulator in plants (Evans and Tramontano, 1981) but does not serve as a biologically available source of niacin.

The paucity of nicotinic acid and the tentative identification of small amounts of nicotinic acid adenine dinucleotide and large amounts of NAD in milky grain suggest that the quinolinic acid pathway for synthesis of NAD is operative in developing corn grain as in other plant tissues (Wagner and Wagner, 1985). Other nicotinic acid derivatives in grain are formed from NAD during development.

Evidence for the biological utilization of NAD as a niacin source was obtained in rat-feeding experiments to be reported elsewhere (Carpenter and Wall, 1987). In those studies, sweet and field corn harvested at different stages of maturity were fed to rats, and their ability to support growth as part of a niacin-deficient diet was determined. Results were consistent with the analytical data in these studies in that mature grains of either field or sweet corns, which contained significant levels of NAD and NADP, supported growth better than diets containing mature field or sweet corn, in which the predominant nicotinic acid containing substances were trigonelline or bound nicotinic acid.

Registry No. NAD, 53-84-9; NADP, 53-59-8; NMN, 1094-61-7; NAAD, 6450-77-7; niacin, 59-67-6; nicotinamide, 98-92-0; trigonelline, 535-83-1.

LITERATURE CITED

AOAC *Official Methods of Analysis of the Association of Official Analytical Chemists*, 13 ed.; AOAC: Washington, DC, 1980; pp 743–746.
Beigenhertz, G.; Bucher, J.; Garbade, K.-H. *Methods Enzymol.* **1955**, *1*, 391–397.
Carpenter, K. J. *Fed. Proc.* **1981**, *40*, 1531.
Carpenter, K. J.; Wall, J. S. *J. Nutr.* **1987**, in preparation.
Carter, E. G. A.; Carpenter, K. J. *Nutr. Res.* **1981**, *1*, 571.
Carter, E. G. A.; Carpenter, K. J. *J. Nutr.* **1982**, *112*, 2091.
Christianson, D. D.; Wall, J. S.; Cavins, J. F. *J. Agric. Food Chem.* **1966**, *13*, 272.
Christianson, D. D.; Wall, J. S.; Dimler, R. J.; Booth, A. N. *J. Agric. Food Chem.* **1968**, *16*, 100.
Evans, L. S.; Tramontano, W. A. *Am. J. Bot.* **1981**, *68*, 1282.
Harper, A. E.; Punekar, B. D.; Elvehjem, C. A. *J. Nutr.* **1958**, *66*, 163.
Kodicek, E. *Biochem. J.* **1940**, *34*, 724.
Kodicek, E.; Braude, R.; Kon, S. K.; Mitchell, K. G. *Br. J. Nutr.* **1956**, *10*, 51.
Mason, J. B.; Kodicek, E. *Cereal Chem.* **1973**, *50*, 637.
Mason, J. B.; Gibson, N.; Kodicek, E. *Br. J. Nutr.* **1973**, *30*, 297.
Miksic, J. R.; Brown, P. B. *J. Chromatogr.* **1977**, *142*, 641.
Payne, S. M.; Ames, B. N. *Anal. Biochem.* **1982**, *133*, 151.
Pearson, W. N.; Stemptel, S. H.; Valenzuela, J. S.; Utley, M. H.; Darby, W. J. *J. Nutr.* **1957**, *62*, 445.
Wagner, R.; Wagner, K. G. *Planta* **1985**, *165*, 532.
Wynants, J.; Van Belle, H. *Anal. Biochem.* **1985**, *144*, 258.

Received for review October 9, 1986. Accepted May 11, 1987. Mention of firm names or trade products does not imply their endorsement by the U.S. Department of Agriculture over others not cited.

Control of the Authenticity of Orange Juice by Isotopic Analysis

J. Bricout* and J. Koziet

Control of the authenticity of orange juices involves the detection of undeclared addition of sugar. The $^{13}C/^{12}C$ ratio of sugar from orange juice is lower than the $^{13}C/^{12}C$ ratio in cane or corn sweetener but similar to the $^{13}C/^{12}C$ ratio in beet sugar. For this reason, we have determined the $^{2}H/^{1}H$ ratio of nonexchangeable carbon-bound hydrogen in sugar from orange and beet. The difference in $\delta(^{2}H)$ of these two sources of sugars is quite large and reflects probably the difference of climate in which these two plants grow.

In Western Europe, orange juices are generally prepared by redilution of concentrates imported from different countries of tropical or subtropical areas. It is necessary to have the ability to verify the purity of orange concentrate and to detect adulteration. Many analytical and statistical methods have been proposed for this purpose (Bielig et al., 1984; Richard and Coursin, 1980; Vandercook et al., 1983); generally, the concentrations of different elements are measured and those results compared to a data base, originally established by analyzing pure juices of different origins. This comparison can be made by different statistical tests (Brown and Cohen, 1983). However, the analytical parameters of oranges are generally not unique but are substances that occur in many types of fruits and vegetables, and are available as cheap chemicals.

In recent years, it was suggested that the isotopic composition of different constituents of our food can be used for the determination of their origin (Bricout, 1982). For example, the isotopic ratio $^{13}C/^{12}C$ can be used to detect the addition of synthetic vanillin to vanilla extract (Bricout et al., 1974) or of high-fructose corn syrup or cane sugar to honey (White and Doner, 1978), apple juice (Doner et al., 1980), and orange juice (Doner and Bills, 1980).

The $^{18}O/^{16}O$ and $^{2}H/^{1}H$ ratios of water allow a confident distinction between natural fruit juices (particularly orange) and reconstituted juices (Bricout, 1973). The enrichment in ^{2}H and ^{18}O of plant water as compared to rain and ground water is due to isotopic fractionation by evapotranspiration. It was shown that this enrichment is higher when the climate is dry and warm (Lesaint et al., 1974), a climatic situation that occurs in some orange-producing areas. A further extension of this observation was the detection of sugar syrup or pulp wash to orange concentrate by the determination of the $^{18}O/^{16}O$ ratio in the water of orange concentrate (Brause et al. 1984) as sugar syrup and pulp wash as manufactured with ground water.

We can anticipate that the $^{2}H/^{1}H$ and the $^{18}O/^{16}O$ ratios of plant water can influence the isotopic composition of plant organic matter. $^{2}H/^{1}H$ ratios were used to distinguish natural aromatic substances from their synthetic equivalents (Bricout and Koziet, 1978). It was shown that orange sugars have higher $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios than beet sugar (Bricout, 1978). However, some experimental difficulties were encountered during these first analyses. The $^{18}O/^{16}O$ analysis of sugar is difficult and the $^{2}H/^{1}H$ ratio of sugar is not constant due to an isotopic exchange between water and hydrogen bound to oxygen. This difficulty can be overcome by nitration, which removes all the exchangeable hydroxyl hydrogen atoms. This method was first developed for cellulose analysis (Epstein et al., 1976) and was extended for grape sugar (Dunbar et al., 1983). The results obtained on plant cellulose have shown a low sensitivity of $^{18}O/^{16}O$ ratio of cellulose to source water (Epstein et al., 1977).

For these reasons, we have examined the $^{2}H/^{1}H$ ratio in nitrated sugars from oranges of known origin and in

Centre de Recherche Pernod Ricard, 120 Avenue Foch, 94015 Creteil Cedex, France.

0021-8561/87/1435-0758$01.50/0