Determination of Vitamin B₆, Available Lysine, and ε-Pyridoxyllysine in a New Instant Baby Food Product

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Selected jar and instant baby foods processed in 1985 and 1987 were analyzed for their vitamin B₆, available lysine, and ε-pyridoxyllysine content in summer 1988. Jar and instant vegetable and beef were found to be higher in available lysine content but lower in vitamin B₆ than banana products. Instant products were found to be higher in vitamin B₆ than jar products. On a wet weight basis the instant products also contained higher amounts of available lysine than jar products. However, after adjustment for protein content, jar products were higher in available lysine than instant products. Baby foods processed in 1985 tended to be lower in vitamin B₆ and available lysine content than products processed in 1987. Pyridoxyllysine was below detectable quantities in all of the baby foods analyzed in this study.

INTRODUCTION

Vitamin B₆ and lysine are two essential nutrients in the human diet and of major importance in the normal development of infants. Vitamin B₆ and available lysine have been determined for many foods, and compilations of data on vitamin B₆ are available (Orr, 1969). Considerable research has also focused on the bioavailability of these nutrients from foods, and mechanisms have been proposed to account for decreases in bioavailability due to thermal processing. The thermal degradation of lysine has been extensively studied (Bender, 1972), whereas less research has been conducted to establish mechanisms for vitamin B₆ degradation (Gregory et al., 1986).

While thermal processing is known to decrease the content of vitamin B₆ in foods (Gregory and Kirk, 1978a; Navankasattusas and Lund, 1982; Hassinen et al., 1954; Schroeder, 1971; Evans et al., 1981), its effect on vitamin B₆ bioavailability has not been fully established. Some researchers have reported decreasing vitamin B₆ bioavailability due to thermal processing (Tarr et al., 1981; Tomarelli et al., 1955), while others have found that thermal processing has little or no effect on B₆ bioavailability (Gregory and Kirk, 1978a; Gregory, 1980a; Ink et al., 1986). It has been suggested that the effects of thermal processing on vitamin B₆ bioavailability are closely related to food composition, i.e., relative amounts of B₆ vitamers, lysine and free sulfhydryl groups in protein, reducing sugars, etc. (Gregory, 1980a; Nguyen and Gregory, 1983).

The bioavailability of vitamin B₆ in foods is believed to be affected by the formation of bis(4-pyridoxyl) disulfide (Srncova and Davidek, 1972), pyridoxine β-glucoside (Kabir et al., 1983b), and ε-pyridoxyllysine (Gregory and Kirk, 1977). While bis(4-pyridoxyl) disulfide is thought to be of only minor importance to B₆ bioavailability (Gregory and Kirk, 1977; Gregory et al., 1986), it has been found that upon thermal processing and storage up to 44% of pyridoxal phosphate can be bound in the form of ε-pyridoxyllysine (Gregory and Kirk, 1978b,c). Small amounts of ε-pyridoxyllysine have been shown to have antivitamin activity attributed in part to competitive inhibition of pyridoxal kinase (EC 2.7.1.35) (Gregory,

1980b,c). This antivitamin activity of ε-pyridoxyllysine could be potentially deleterious to nutritional status if the diet is otherwise deficient in vitamin B₆ (Gregory, 1980c). However, to date, ε-pyridoxyllysine formation has been demonstrated only in model food systems and a few processed foods high in protein (Gregory and Kirk, 1981; Gregory et al., 1986).

In the early 1950s a newly introduced thermal processing method rendered an infant formula almost devoid of bioavailable vitamin B₆, leading to convulsive seizures and some fatalities in young infants (Snyderman et al., 1953). Recently, a new instant baby food was introduced to the U.S. market. This new instant baby food is processed by drum-drying, a drying method which may result in considerable nutrient destruction (Erbersdobler, 1986).

The objectives of this study, therefore, were to determine (1) if there are significant differences in the vitamin B₆, available lysine, and pyridoxyllysine content of similar jar and instant baby foods and (2) if significant differences in vitamin B₆ and available lysine content of baby foods are due to product formulation or processing year.

MATERIALS AND METHODS

Materials. Instant baby foods of the product formulations "Vegetable and Beef" and "Bananas" and their matching jar products were kindly donated to us by H. J. Heinz Co., Pittsburgh, PA, in winter 1985. Both instant and jar products were processed at about the same time and, upon arrival in our laboratory, were stored in the dark at room temperature. Similar instant and jar baby foods processed in 1987 were purchased in spring 1988 from a local supermarket. The instant baby foods were reconstituted according to label directions prior to analyses in summer 1988.

ε-Pyridoxyllysine was synthesized according to the method of Dempsey and Christensen (1962) and Dempsey and Snell (1963) with some modifications (Gregory, 1980b; Severin et al., 1969). The method is based on the condensation of α-acetyllysine (Aldrich Chemical Co., Milwaukee, WI) with pyridoxal hydrochloride (Sigma Chemical Co., St. Louis, MO) followed by reduction with sodium borohydride forming α-acetyl-ε-pyridoxyllysine. Salts and unreacted material were removed by column chromatography using a Sephadex G-10 column (2.6 × 60 cm) (Pharmacia Fine Chemicals, Piscataway, NJ). Acid hydrolysis of the acetyl moiety yielded ε-pyridoxyllysine, which was purified by ion-exchange chromatography on Bio-Rex 70 (Bio-Rad Laboratories, Richmond, CA). The identity of the synthesized pyridoxyllysine was verified by mass spectrometry and UV–visible spectroscopy.

Methods. *Vitamin B₆ Determination.* The total vitamin B₆ content of baby food samples was determined microbiologically

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by using AOAC Method 43.229 (AOAC, 1984). The vitamin B$_6$ content of each baby food was determined by analyzing three instant or jar products, subsampled in triplicate.

Crude Protein Determination. The nitrogen content of the baby foods was determined by analyzing four samples of each baby food by using the Kjeldahl AOAC Method 7.015 (AOAC, 1984). The protein content of the baby foods was estimated by multiplying the nitrogen content of the samples by 6.25.

Available Lysine Determination. The available lysine content of each baby food was determined by analyzing four instant or jar products, subsampled in duplicate. The available lysine content of baby food samples was determined by using a trinitrobenzenesulfonic acid (TNBS) method, adapted from Hall et al. (1973). The method was modified according to James and Ryley (1986), Hall and Henderson (1979), Posati et al. (1972), and Hall et al. (1975) to be applicable to carbohydrate-rich foods. The method is based on the reaction of the free ε-amino group with trinitrobenzenesulfonic acid (Sigma), forming trinitrophenyllysine. Trinitrophenyllysine (TNP-L-lysine) is hydrolyzed from the protein and measured spectrophotometrically at 415 nm.

Depending on the protein content of the various product formulations, between 5 and 30 g of baby food (wet weight) was suspended in an agar solution (0.1% w/v). A 0.5-mL aliquot of the vegetable and beef sample suspension (or 1 mL of banana sample suspension) was placed into two 10-mL graduated test tubes, and 0.5 mL of 1 M sodium hydrogen carbonate solution was added to each tube. One milliliter of TNBS (1% w/v in distilled water) was then added to one of the two tubes to let free ε-amino groups of lysine react with TNBS (Hall et al., 1973). This tube was incubated for 30 min at 30 °C (Hall et al., 1975). The reaction was terminated by adding 3 mL of concentrated hydrochloric acid. Three milliliters of concentrated hydrochloric acid was added to the other tube prior to TNBS addition to prevent the reaction of TNBS and lysine. This tube was designated as the sample blank. Sample blanks were needed to subtract the absorbance of the food and reactants at 415 nm that is not due to TNP-L-lysine formation. All tubes were subjected to acid hydrolysis in a vigorously boiling water bath for 1 h (James and Ryley, 1986). After filtering (Posati et al., 1972), 3-mL aliquots from each test tube were pipetted in duplicate into separate test tubes. Free TNBS and other TNP amino acids were removed with ethyl ether. The banana formulations were bleached with hydrogen peroxide (3% v/v) and potassium permanganate (4% w/v) (James and Ryley, 1986; Hall and Henderson, 1979). The absorbance of samples and sample blanks was measured at 415 nm by using a Bausch and Lomb Spectronic 2000 spectrophotometer. The absorbance of the sample blanks was subtracted from the absorbance of the samples, and the corresponding available lysine values were determined by using a standard curve. ε-TNP-L-lysine monohydrochloride monohydrate (ICN Nutritional Biochemical, Cleveland, OH) was used as an external standard in concentrations of 0.6, 1.2, 1.8, 2.4, 3.0, 4.2, and 5.4 μg/mL of lysine to establish the standard curve. The standards were treated identically with the samples. The recovery of these standards was 93–97% when compared to a TNP-L-lysine standard that did not undergo extraction. The data were not adjusted for recovery rates.

Pyridoxyllysine Determination. Pyridoxyllysine was determined by using two different methods. Both methods were done under subdued light. The two methods are based on the ability of pyridoxyllysine to react under certain conditions as an amino acid and also to fluoresce at the same wavelengths as B$_6$ vitamers. The first method was a standard procedure for amino acid analysis using the Pico-Tag system of Waters Associates, Milford, MA. The method is based on acid hydrolysis of the samples with subsequent derivatization of the free amino acids with phenyl isothiocyanate to form phenylthiocarbamyl amino acids. The derivatized amino acids are separated by HPLC and detected spectrophotometrically at 254 nm.

The following procedure was used for acid hydrolysis and derivatization of instant and jar baby food samples. First, 0.1 g of each dried sample was weighed into 20-mL glass ampules and 7.5 mL of 6 N HCl was added to the ampules. The ampules were vortexed, and another 7.5 mL of 6 N HCl was added. The ampules were then sealed and autoclaved at 132 °C for 6 h and then cooled to room temperature. Five milliliters of 515 μg/mL α-aminobu-

tyric acid (internal standard) was added to 25-mL volumetric flasks. The contents of the ampules were poured into the flasks and mixed, and the volume was brought up to 25 mL with distilled deionized water and mixed again. A 1-mL aliquot of each sample was removed by using a tuberculin syringe and passed through a 0.45-μm filter into a small screw-top glass vial. The vial was purged with nitrogen and frozen at -20 °C. The sample was thawed immediately prior to analysis, and a 10-μL aliquot was placed into sample tubes and dried to 70 Torr on the vacuum station. Ten microliters of Redry solution was added to each standard and sample tube and vortexed. The tubes were placed in a reaction vial and dried to 70 Torr. Then 20 μL of derivatization reagent was added to each tube. After vortexing, the tubes were placed into the reaction vial in the vacuum station and the vacuum port was opened after a derivatization time of 20 min. The samples were dried to 70 Torr, and 200 μL of diluent was added to each sample tube and vortexed. The sample tubes were then centrifuged in a microcentrifuge at 1200 rpm for 30 s at room temperature. The supernatant was removed to limited volume inserts and placed in the appropriate WISP vials. The vials were closed with Teflon septa and screw caps and were placed into the automatic sampler.

The HPLC system (Waters Associates, Milford, MA) used for separation of derivatized amino acids consisted of a WISP Model 712, a column temperature control system, two Model 510 solvent delivery systems, a Pico-Tag column (3.9 mm × 15 cm), a Model 441 absorbance detector, and a system interface module that connected the system to a NEC APC IV personal computer. The sample size injected was 10 μL, and the pyridoxyllysine standard had a concentration of 743 μg/mL.

The second method for determining pyridoxyllysine was based on modified procedures of Chrisley et al. (1988) and Gregory et al. (1986). We attempted first to extract pyridoxyllysine and B$_6$ vitamers simultaneously using the procedure of Chrisley et al. (1988), which had been developed for the determination of B$_6$ metabolites in plasma samples. In this method, reversed-phase HPLC with UV and fluorometric detection is used to detect nanogram quantities of the seven common vitamin B$_6$ metabolites in plasma. The HPLC system (Waters Associates) used in this method consisted of a Model 730 data module, a Model 720 system controller, two Model 45 solvent delivery systems, a Model U6K universal injector, a column temperature control system, a Model 440 UV absorbance, and a Model 420 E/AC fluorescence detector (300-nm excitation, 375-nm emission filters). The analytical column was a μBondapak octadecylsilane column (30 cm × 3.9 mm i.d., 10-μm porous packing). The mobile phase for gradient elution consisted of methanol/water (85:15 v/v; solvent A) and the two paired-ion reagents PIC B-7 and B-8 (0.005 M heptanesulfonic acid and octanesulfonic acid in 1% acetic acid; solvent B) with a flow rate of 1 mL/min at ambient temperatures.

Twenty-five microliters of pyridoxyllysine standard was injected into this HPLC system in concentrations of 125, 167, 250, 500, 1000, and 2000 ng/mL, which resulted in a linear standard curve. The 125 ng/mL standard gave a peak 2 times noise level and was considered detectable, while the 167 ng/mL standard resulted in a peak 3 times noise level, which was considered quantifiable. Individual and combined standards of the B$_6$ vitamers were mixed with the pyridoxyllysine standard to confirm the separation of pyridoxyllysine from the B$_6$ vitamers. Peak identities were confirmed by spiking and retention times. For extracting B$_6$ vitamers and pyridoxyllysine from the food, trichloroacetic acid (TCA) was added to diluted food samples to give a final concentration of 5% TCA. The mixture was centrifuged and the supernatant was decanted. The supernatant was to be used for the B$_6$ analysis, and the coagulated protein hydrolyzed to release pyridoxyllysine. After hydrolysis, the hydrolysate was to be purified by evaporation and ion-exchange chromatography according to the method of Gregory et al. (1986). The major problem with this extraction method was that it did not give reproducible results, either for vitamin B$_6$ or for pyridoxyllysine. This was true for both spiked and unspiked food samples. The method was developed for the extraction and determination of B$_6$ vitamers in plasma samples and does not seem to be applicable without further modifications to complex food systems.

In the extraction procedure of Gregory et al. (1986) the sample is dialyzed to reduce simple sugars and free B$_6$ vitamers. The

Table I. Mean[a] Vitamin B₆ (Expressed as Pyridoxine Hydrochloride) Content of Analyzed Baby Foods

sample	$X \pm SD$, nmol/g	$X \pm SD$, mg/100 g
bananas, instant, 1987	10.36 ± 0.83	0.213[a] ± 0.017
bananas, instant, 1985	8.90 ± 0.58	0.183[b] ± 0.012
vegetable and beef, instant, 1987	3.90 ± 0.34	0.081[c] ± 0.007
vegetable and beef, instant, 1985	3.65 ± 0.19	0.075[c] ± 0.004
bananas, jar, 1987	3.89 ± 0.15	0.080[c] ± 0.003
bananas, jar, 1985	3.50 ± 0.19	0.072[c] ± 0.004
vegetable and beef, jar, 1987	2.72 ● 0.10	0.056[d] ± 0.002
vegetable and beef, jar, 1985	2.58 ± 0.19	0.053[d] ± 0.004

[a] $N = 3$ in triplicate. Means with the same letter are not significantly different at $\alpha = 0.05$.

contents of the dialysis bag is then subjected to acid hydrolysis. Various modifications of this extraction method were tried. The sample sizes of baby food extracted varied among 1, 2, 4, and 20 g. Due to the high carbohydrate content of the baby food samples, a black "charring" product formed during acid hydrolysis and was removed by filtration of the sample through Whatman No. 44 filter paper. After evaporation, washing, and re-evaporation, the samples were subjected to ion-exchange chromatography using Bio-Rex 70 (Bio-Rad Laboratories) as described by Gregory et al. (1986) for liver and muscle hydrolysates. Since we were able to separate and detect pyridoxyllysine with the HPLC system of Chrisley et al. (1988), the evaporated eluate was taken up in 2 mL of HPLC solvent B, and 25 μL of this solution was injected into the HPLC system as described earlier. The following modifications were made in Gregory's extraction procedure in an attempt to detect and quantify pyridoxyllysine: (1) the enzymes α-amylase and amyloglucosidase (Sigma) were added to the dialysis bag to break down complex carbohydrates into dialyzable saccharides, (2) straight acid hydrolysis without prior dialysis of the samples, and (3) direct analysis of hydrolyzed samples without prior ion-exchange chromatography.

Total Lysine Determination. The amount of total lysine in the baby food formulations was determined by using amino acid analysis as described above.

Statistical Analysis. Analysis of variance and Duncan's multiple range test of the Statistical Analysis System (SAS Institute, 1985) were used to determine significant differences in the vitamin B₆ and available lysine content of instant and jar baby foods.

RESULTS

Vitamin B₆. The coefficient of variation for the determination of vitamin B₆ was 0.14, established by analyzing six identical samples in triplicate. Percent recoveries of B₆ vitamers ranged from 93% to 112% for PN, from 61% to 69% for PL, and from 93% to 98% for PM; vitamer recoveries were similar in all baby foods. The results of the vitamin B₆ analysis are given in Table I.

Banana products were found to be significantly higher in vitamin B₆ content than vegetable and beef products. Instant products were significantly higher in vitamin B₆ content than jar products. Except for the instant banana product, no significant differences were found in the vitamin B₆ content of similar 1985 and 1987 products.

Protein. The results of the protein determination expressed as percent protein are given in Tables II and III. The protein content of the instant products was significantly higher than that of the jar products.

Total Lysine. The results of the total lysine analysis are shown in Tables II and III.

Available Lysine. The coefficient of variation for the available lysine determination was 0.054, established by analyzing five identical samples in duplicate. The results of the available lysine analysis were expressed in milligrams of lysine per 100 g of food, and when adjusted for protein content, as milligrams of lysine per gram of nitrogen (N).

Statistical analysis of mean available lysine values was conducted separately on the two product formulations; otherwise, large differences between vegetable and beef and banana formulations would tend to mask smaller yet significant differences between instant and jar bananas or between 1985 and 1987 bananas. Results for vegetable and beef are shown in Table II, and the results for the bananas products are presented in Table III.

When the results were expressed in milligrams per 100 g of food, the instant products were found to contain significantly more protein and available lysine than jar products. However, jar products were significantly higher in available lysine than instant products when results were adjusted for protein content and expressed on a milligram per gram of N basis. Except for the jar banana products, there were no significant differences in the available lysine content of 1985 and 1987 products.

Pyridoxyllysine. Pyridoxyllysine was not found in detectable quantities in any of the baby foods analyzed in this study. The pyridoxyllysine standard, however, was detectable both by amino acid analysis and by HPLC. The peak of the pyridoxyllysine standard was well resolved in both analyses (see Figures 1 and 2). While 20-g samples overloaded the HPLC column and resulted in poor resolution of pyridoxyllysine from spiked samples, chromatograms from 4-g samples showed a well-resolved peak for the pyridoxyllysine spike (Figure 3) but no indication that the baby food contained pyridoxyllysine (Figure 4). The detection limit of the instrument was 125 ng/mL or 0.42 nmol/mL. The injection size was 25 μL; thus, 3 ng or 10 pmol of pyridoxyllysine was detectable, which gave peaks 2 times noise level. Considering a sample size of 4 g was extracted into 2 mL and the detection limit of the instrument was 125 ng/mL or 0.42 nmol/mL, it must be concluded that pyridoxyllysine is below the concentration of 62.5 ng or 0.21 nmol/g of food.

There may be several reasons why we could not detect pyridoxyllysine in the baby food. The most likely reason is that pyridoxyllysine is present in quantities that are below the detection limits of the methods employed in this study. Gregory and Kirk (1978b,c) reported that 44% of pyridoxal phosphate could be lost in the form of bound pyridoxyllysine during thermal processing and storage. In an earlier study, only 10% of the total PLP was found to be bound in a nonreducible form, considered to be pyridoxyllysine (Gregory and Kirk, 1977). In a more recent study with model food systems, Gregory et al. (1986) used a vitamin B₆ content comparable to endogenous levels found in foods and detected up to 25% of pyridoxal phosphate/pyridoxal bound in the form of pyridoxyllysine. Pyridoxal phosphate is probably the lowest of the three major B₆ vitamers (pyridoxine, pyridoxamine phosphate, pyridoxal phosphate) present in the baby foods analyzed in this study and can be estimated to be about 10% of the total B₆ in the banana products and 30% in the vegetable and beef (Orr, 1969) products. Thus, if the 44% figure reported by Gregory et al. (1978b,c) were applicable to these baby foods, about 10.6 μg (35.7 nmol) of pyridoxyllysine/100 g of food could be expected for instant vegetable and beef, 8.8 μg (29.6 nmol)/100 g for instant bananas, 8 μg (26.9 nmol)/100 g for jar vegetable and beef, and 3.5 μg (11.8 nmol)/100 g for jar bananas. Sample sizes of 1, 2, 4, and 20 g were used, which were extracted into 2 mL of solvent. A 1-g sample of instant vegetable and beef would result in 53.5 ng (0.18 nmol) of pyridoxyllysine/mL of extract. Since the lowest detectable limit of pyridoxyllysine in our HPLC system is 125 ng/mL (0.42 nmol/mL) and the lowest quantifiable limit is

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Table II. Mean Total Lysine, Available Lysine, and Protein Content of Vegetable and Beef Products

sample	total lysine,[a] mg/g of N	available lysine[b,c] mg/100 g of food ± SD	available lysine[b,c] mg/g of N ± SD	protein,[c] % ± SD
instant, 1987	393.7	246.4[a] ± 9.4	384.8[bc] ± 14.8	3.85[b] ± 0.15
instant, 1985	393.2	244.3[a] ± 16.3	381.5[c] ± 25.4	4.10[a] ± 0.04
jar, 1987	441.3	181.9[b] ± 8.6	437.0[a] ± 20.7	2.56[c] ± 0.08
jar, 1985	438.1	173.1[b] ± 9.8	416.3[ab] ± 23.8	2.66[c] ± 0.04

[a] N = 1 in duplicate. [b] N = 4 in duplicate. [c] Means with the same letter are not significantly different at α = 0.05.

Table III. Mean Total Lysine, Available Lysine, and Protein Content of Bananas Products

sample	total lysine,[a] mg/g of N	available lysine[b,c] mg/100 g of food ± SD	available lysine[b,c] mg/g of N ± SD	protein,[c] % ± SD
instant, 1987	440.1	24.7[a] ± 1.4	237.5[c] ± 13.6	0.69[a] ± 0.04
instant, 1985	453.7	23.0[a] ± 1.1	221.3[c] ± 10.6	0.61[a] ± 0.02
jar, 1987		18.4[b] ± 2.4	382.8[a] ± 49.9	0.32[b] ± 0.01
jar, 1985		13.7[c] ± 1.4	286.3[b] ± 28.6	0.31[b] ± 0.02

[a] N = 1 in duplicate. [b] N = 4 in duplicate. [c] Means with the same letter are not significantly different at α = 0.05.
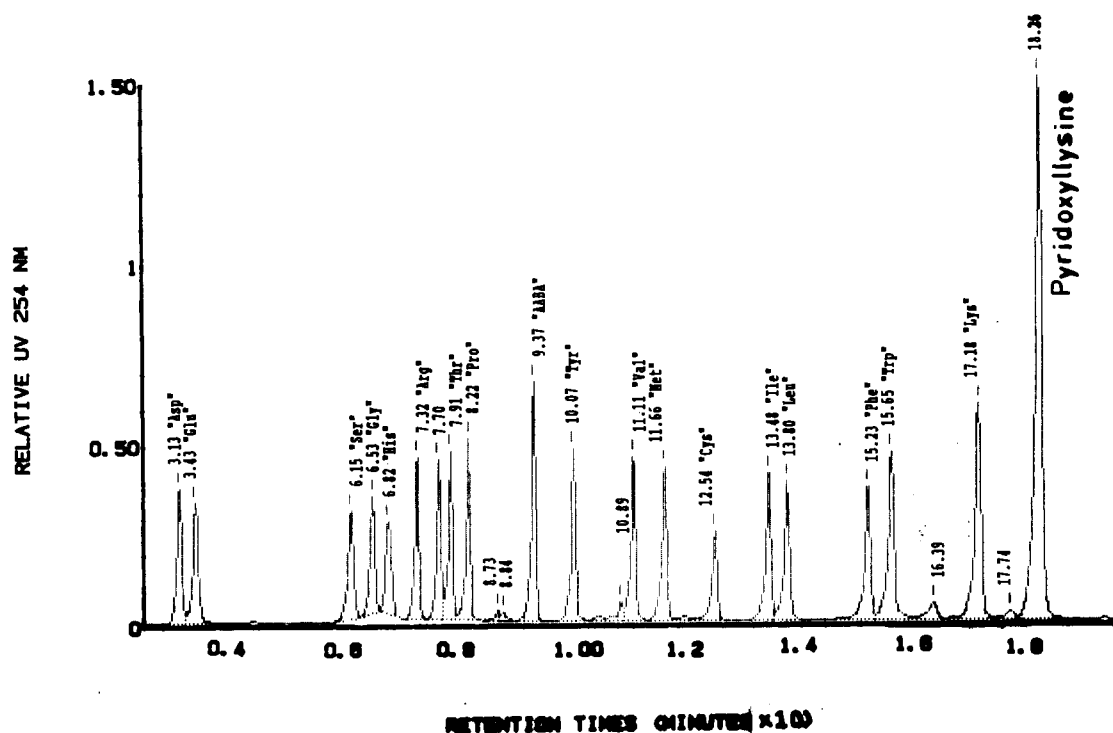

Figure 1. Pyridoxyllysine standard separated from amino acid standards by amino acid analysis.

167 ng/mL (0.56 nmol/mL), a 2-g sample would have contained detectable amounts and a 4-g sample quantifiable amounts of pyridoxyllysine if the 44% figure were applicable to these foods.

Although these calculations do not provide conclusive evidence that pyridoxyllysine is below detection limits in the baby food, it does suggest that pyridoxal/pyridoxal phosphate might have not interacted with lysine to form pyridoxyllysine to the extent reported by Gregory and Kirk in 1978 (1978b,c) (44%) but perhaps rather to the extent reported in 1977 (10%) or in 1986 (10-25%) (Gregory and Kirk, 1977; Gregory et al., 1986). It is important to note that these results are not in disagreement with the results reported by these researchers (Gregory et al., 1986; Gregory and Kirk, 1981) because the foods and model food systems used in those studies are high in protein, whereas the baby foods are relatively low in protein. In addition, Gregory and Kirk (1977) have suggested that Maillard browning may retard pyridoxyllysine formation, and the baby foods, especially the banana products, are relatively high in reducing sugars (Souci et al., 1986).

Another possible reason pyridoxyllysine was not detected might be because it was not completely released from food proteins but remained bound to small peptides. However, this possibility is unlikely because 48-h acid hydrolysis should have cleaved all peptide bonds and released any pyridoxyllysine originally part of proteins. A third possibility worth considering is that pyridoxyllysine forms a complex with some other food components after being released from hydrolyzed protein. If so, then the question arises, why should the pyridoxyllysine in the baby food undergo this complex formation and not pyridoxyllysine added in the form of spikes? The recovery of the spikes was always between 95% and 100%. A fourth consideration is the stability of the pyridoxyllysine. Although initially Gregory and Kirk (1978c) reported good stability of pyridoxyllysine under acid hydrolysis conditions, they later found a 20-25% destruction of pyridoxyllysine (Gregory et al., 1986). In preliminary studies we determined pyridoxyllysine degradation under acid hydrolysis conditions using UV spectrophotometry. We detected little or no pyridoxyllysine destruction under these conditions, which is in agreement with Polyanovskii (1963),
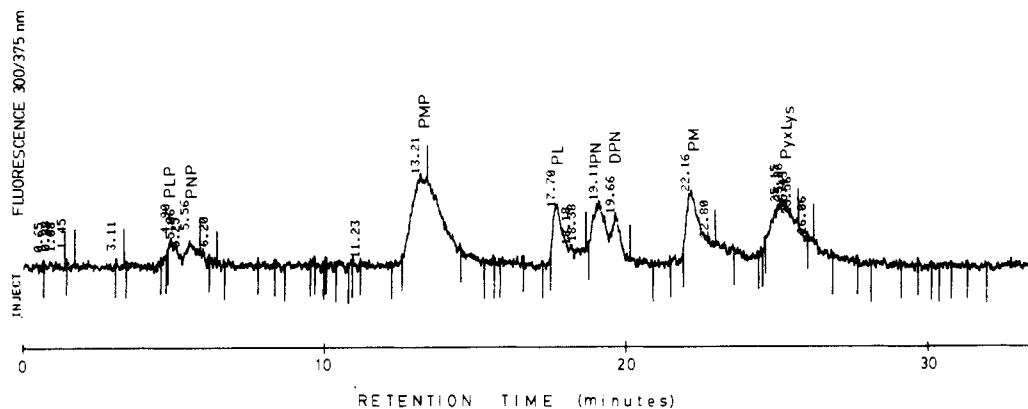
Figure 2. Pyridoxyllysine standard separated from B6 vitamer standards by HPLC analysis. (PLP = pyridoxal phosphate, PNP = pyridoxine phosphate, PMP = pyridoxamine phosphate, PL = pyridoxal, PN = pyridoxine, PM = pyridoxamine, DPN = 4-deoxypyridoxine, PyxLys = ε-pyridoxyllysine.)
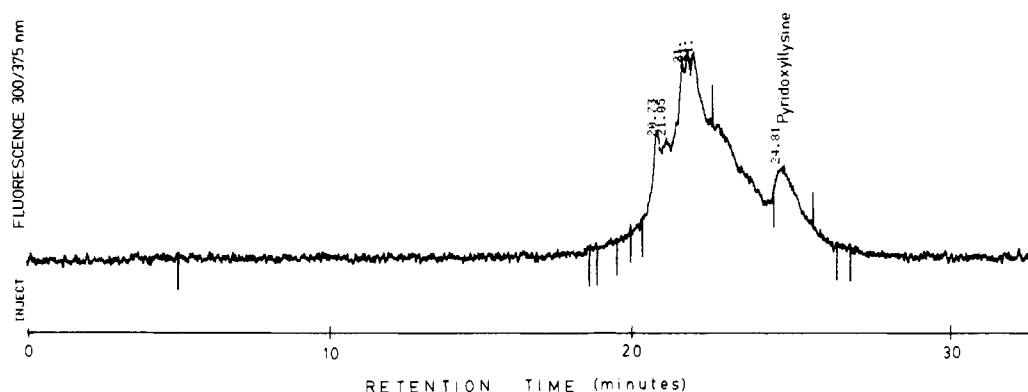

Figure 3. HPLC chromatogram of 4 g of instant vegetable and beef sample with pyridoxyllysine spike (500 ng/mL).
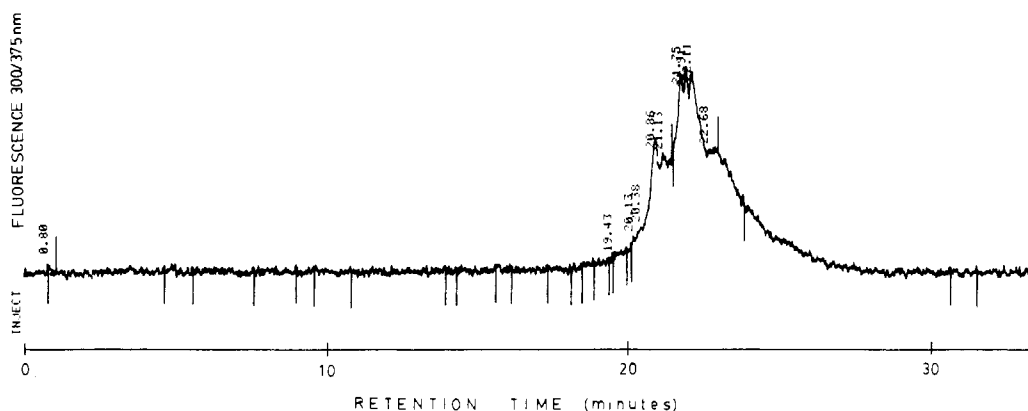

Figure 4. HPLC chromatogram of 4 g of instant vegetable and beef sample without pyridoxyllysine spike.

who did not detect any significant destruction after 72 h of hydrolysis. In addition, we observed 95–100% recovery of pyridoxyllysine in spiked baby food samples when compared to the pyridoxyllysine standard.

DISCUSSION

Vitamin B6. It is difficult in the case of vitamin B6 to make any statement about the effect of the two processing methods because it is not possible to relate it to a relative term. In fact, it is not possible to separate a processing effect from a "dilution" effect caused by the addition of various ingredients such as starches with little or no vitamin B6 in both the jar banana and the jar vegetable and beef product. Thus, it is not possible to directly determine whether differences in the vitamin B6 content of instant and the jar products are due to processing alone or due

also to differences in formulation of the instant and jar products. In addition, the label of the jar banana product specifies "fully ripened bananas". Hardin et al. (1989) reported that the vitamin B6 content of bananas increases during the ripening process. Thus, if fully ripened bananas were used for jar but not for instant products, a difference in vitamin B6 content would have been introduced, making the original jar products higher in B6 than instant products. Thus, we can conclude only that the instant products were higher in vitamin B6 content, but the reasons are uncertain.

Various investigators have shown that vitamin B6 can be found in the conjugated form of pyridoxine β-glucoside in foods of plant origin (Kabir et al., 1983a; Gregory and Ink, 1987). It has been shown that vitamin B6 bioavailability is inversely related to the level of this compound in food (Kabir et al., 1983b). Since this study did not

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determine pyridoxine β-glucosides, the total vitamin B_6 content of the baby food is probably higher than that determined by microbiological assay. Bananas have been reported to contain 3–5% glycosylated vitamin B_6 (Kabir et al., 1983a; Gregory and Ink, 1987); vegetable and beef products probably contain smaller amounts of mono- and disaccharides and thus less B_6 glucosides.

The vitamin B_6 content of fresh bananas is reported to be between 0.37 and 0.51 mg/100 g (Souci et al., 1986; Hardin et al., 1989; Orr, 1969); thus, a B_6 content of approximately 0.2 mg/100 g of food for instant bananas, which consists only of bananas, indicates that there may be some vitamin B_6 degradation due to drum-drying. However, it is not possible to calculate percent loss of vitamin B_6 due to processing because the vitamin B_6 content of the raw material is not known. It is also not possible to consider vitamin B_6 losses due to drum-drying for the jar or instant vegetable and beef product or the jar banana product because of the addition of various food ingredients. However, the vitamin B_6 contents of the ingredients for the vegetable and beef products are reported in the literature, and some inferences can be made by using these values. The vitamin B_6 content of canned carrots is reported to be between 0.022 (Souci et al., 1986) and 0.03 mg/100 g of food (Orr, 1969), canned tomatoes contain 0.09 mg/100 g, canned peas contain 0.02 mg/100 g, and beef (strained baby food) contains 0.2 mg/100 g vitamin B_6 (Orr, 1969). Thus, the vitamin B_6 contents of the analyzed baby foods appear to be within reported ranges for their component ingredients.

The RDA for infants 0–0.5 year of age is 0.3 mg of vitamin B_6 per day (Food and Nutrition Board, 1989). In evaluating the products in regard to vitamin B_6 supply to the infant, we need to consider the serving sizes of the products, which are 100 g for instant formulations and 128 and 134 g for the jar vegetable beef and banana, respectively. Thus, a serving of instant bananas supplies approximately 70%, jar bananas and instant vegetable and beef approximately 26%, and jar vegetable and beef approximately 18% of the 1989 RDA of vitamin B_6 for this age group.

Total and Available Lysine. While total lysine content of the vegetable and beef product is only slightly higher than the available lysine content, there is a considerable difference for the banana product, which can probably be attributed to greater formation of Maillard browning products in the banana products due to a higher content of reducing sugars.

Instant products were found to be higher in available lysine per 100 g of food than jar products, which is most likely due to their higher protein content. It is necessary to express the available lysine content on the basis of protein content to be able to compare the product formulations and processing methods. The jar products are higher in available lysine when the results are expressed in terms of milligrams of available lysine per gram of nitrogen. Under the assumption that the protein composition is similar for the instant and the jar products, it can be concluded that drum-drying, a method that heats the food to a higher temperature than retorting, is more detrimental in regard to the available lysine content than the canning procedure. These results are in general agreement with those of Dexter et al. (1984), who demonstrated that lysine bioavailability in foods decreases as a function of the processing temperature. The above assumption can be safely made for the banana products because bananas are the only ingredients containing protein in this product. In the case of the vegetable and beef products we have a mixture of protein sources; thus, differences in the available lysine content of instant and jar products may also be due to differences in product formulation.

In regard to storage, while the results are not statistically significant, there is a tendency for 1985 products to be lower in available lysine than 1987 products. This tendency is stronger for the banana products and the jar products. Horvatic and Grüner (1984) also found a small, 6.9%, loss of available lysine in seven different infant formulas over a 12-month storage period.

It is again necessary to consider serving sizes when the available lysine content of the baby foods is evaluated. According to product labels, a single serving consists of 100 g of the instant formulation and 128 and 134 g of jar vegetable and beef and bananas, respectively. Thus, one serving of instant bananas supplies an infant with about 25 mg of available lysine, and one serving of instant vegetable and beef supplies 250 mg of available lysine. Since the serving sizes for the jar products are larger than that of the instant products, the amount of lysine available to the infant is about the same for the jar products, 25 mg for the banana and 230 mg for the vegetable and beef formulations. The estimated lysine requirement for 3–4-month-old infants is 103 mg of lysine per kilogram of body weight per day (Food and Nutrition Board, 1989). Assuming a body weight of 5 kg, one serving of instant vegetable and beef will supply approximately 49%, the jar vegetable and beef 45%, and the banana products 4.9% of the RDA for this age group. Since the banana products can be considered a dessert or snack, while the vegetable and beef products are considered full dinners, these results indicate that these baby foods provide an adequate supply of available lysine to infants.

Pyridoxyllysine. Small concentrations of pyridoxyllysine may be deleterious to vitamin B_6 status when the diet is otherwise deficient in vitamin B_6, while in higher amounts the vitamin B_6 activity of pyridoxyllysine overcomes its antivitamin activity. Symptons of vitamin B_6 deficiency developed more rapidly in rats when pyridoxyllysine was present in the diet at ≤ 0.5 μg of pyridoxine equivalents per gram of food than when rats were fed a diet totally deficient in B_6 (Gregory, 1980c). Gregory (1980c) also reported that vitamin B_6 deficiency symptoms were readily overcome by the presence of 0.5 μg of pyridoxine per gram of diet. It was not possible to detect pyridoxyllysine in any of the baby foods with 250 ng/4 g of food being the detection limit of our instrumentation, while all the baby foods were found to contain adequate levels of B_6. Therefore, while the foods under study are most probably below the pyridoxyllysine level reported to aggravate deficiency symptoms in rats, they do contain high enough levels of vitamin B_6 to prevent deficiencies. Thus, there is little or no reason for concern that any pyridoxyllysine possibly present in the instant or jar baby foods might impair vitamin B_6 status when fed to infants.

SUMMARY AND CONCLUSIONS

The results of this study indicate that drum-drying appears to be more detrimental to available lysine and possibly vitamin B_6 content than retorting. However, all products seem to provide sufficient amounts of the nutrients under study when part of a complete diet. Products processed in 1985 tend to be lower in vitamin B_6 and available lysine than products processed in 1987, which might reflect nutrient losses during storage but could also be caused by differences in the nutrient content of raw materials. Pyridoxyllysine was not detected in the baby

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foods and, therefore, does not appear to influence vitamin B_6 bioavailability in these foods. Further research should focus on the development of suitable methodologies to detect and quantify pyridoxyllysine in processed food products, specifically carbohydrate-rich foods.

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LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Washington, DC, 1984.

Bender, A. E. Processing damage to protein food. A review. *J. Food Technol.* 1972, *7*, 239–250.

Chrisley, B.; Thye, F. W.; McNair, H. M.; Driskell, J. A. Plasma B-6 vitamer and 4-pyridoxic acid concentrations of men fed controlled diets. *J. Chromatogr.* 1988, *428*, 35–42.

Dempsey, W. B.; Christensen, H. N. The specific binding of pyridoxyl 5′-phosphate to bovine plasma albumin. *J. Biol. Chem.* 1962, *237*, 1113–1120.

Dempsey, W. B.; Snell, E. E. Pyridoxamine-pyruvate transaminase. II. Characteristics of the enzyme. *Biochemistry* 1963, *2*, 1414–1419.

Dexter, J. E.; Tkachuk, R.; Matsuo, R. R. Amino acid composition of spaghetti: Effect of drying conditions on total and available lysine. *J. Food Sci.* 1984, *49*, 225–228.

Erbersdobler, H. F. Loss of nutritive value on drying. In *Concentration and Drying*; McCarthy, D., Ed.; Elsevier Science Publishing: New York, 1986; pp 69–87.

Evans, S. R.; Gregory III, J. F.; Kirk, J. R. Thermal degradation kinetics of pyridoxine hydrochloride in dehydrated model food systems. *J. Food Sci.* 1981, *40*, 555–558, 563.

Food and Nutrition Board. *Recommended dietary allowances*, 10th ed.; National Academy of Sciences, National Academy Press: Washington, DC, 1989.

Gregory III, J. F. Bioavailability of vitamin B-6 in nonfat dry milk and a fortified rice breakfast cereal product. *J. Food Sci.* 1980a, *45*, 84–86, 114.

Gregory III, J. F. Effects of epsilon-pyridoxyllysine and related compounds on liver and brain pyridoxal kinase and liver pyridoxamine (pyridoxine) 5′-phosphate oxidase. *J. Biol. Chem.* 1980b, *255*, 2355–2359.

Gregory III, J. F. Effects of epsilon-pyridoxyllysine bound to dietary protein on the vitamin B-6 status of rats. *J. Nutr.* 1980c, *110*, 995–1005.

Gregory III, J. F.; Ink, S. L. Identification and quantification of pyridoxine-β-glucoside as a major form of vitamin B_6 in plant-derived foods. *J. Agric. Food Chem.* 1987, *35*, 76–82.

Gregory III, J. F.; Kirk, J. R. Interaction of pyridoxal and pyridoxal phosphate with peptides in a model food system during thermal processing. *J. Food Sci.* 1977, *42*, 1554–1557.

Gregory III, J. F.; Kirk, J. R. Assessment of roasting effects on vitamin B-6 stability and bioavailability in dehydrated food systems. *J. Food Sci.* 1978a, *43*, 1585–1589.

Gregory III, J. F.; Kirk, J. R. Assessment of storage effects on vitamin B-6 stability and bioavailability in dehydrated food systems. *J. Food Sci.* 1978b, *43*, 1801–1808.

Gregory III, J. F.; Kirk, J. R. Vitamin B-6 activity for rats of pyridoxyllysine bound to dietary protein. *J. Nutr.* 1978c, *108*, 1192–1199.

Gregory III, J. F.; Kirk, J. R. The bioavailability of vitamin B_6 in foods. *Nutr. Rev.* 1981, *39*, 1–8.

Gregory III, J. F.; Ink, S. L.; Sartain, D. B. Degradation and binding to food proteins of vitamin B-6 compounds during thermal processing. *J. Food Sci.* 1986, *51*, 1345–1351.

Hall, R. J.; Henderson, K. An improvement in the determination of available lysine in carbohydrate-rich material. *Analyst* 1979, *104*, 1097–1100.

Hall, R. J.; Trinder, N.; Givens, D. I. Observations on the use of 2,4,6-Trinitrobenzenesulphonic acid for the determination of available lysine in animal protein concentrates. *Analyst* 1973, *98*, 673–686.

Hall, R. J.; Trinder, N.; Wood, M. R. The determination of available lysine in carbohydrate-rich material. *Analyst* 1975, *100*, 68–70.

Hardin, K.; Ridlington, J.; Leklem, J. Increased vitamin B-6 content in ripening bananas. *FASEB J.* 1989, *3* (3), 668, Abstract 2569.

Hassinen, J. B.; Durbin, G. T.; Bernhardt, F. W. The vitamin B_6 content of milk products. *J. Nutr.* 1954, *53*, 249–257.

Horvatic, M.; Grüner, M. Effect of storage on available lysine in dietetic products based on milk. *Hrana Ishrana* 1984, *25* (5/6), 99–101; *Nutr. Abstr. Rev. Ser. A* 1986, *56* (9), 618, No. 5409.

Ink, S. L.; Gregory III, J. F.; Sartain, D. B. Determination of vitamin B_6 bioavailability in animal tissue using intrinsic and extrinsic labeling in the rat. *J. Agric. Food Chem.* 1986, *34*, 998–1004.

James, N. A.; Ryley, J. The rapid determination of chemically reactive lysine in presence of carbohydrates by a modified trinitrobenzenesulfonic acid procedure. *J. Sci. Food Agric.* 1986, *37*, 151–156.

Kabir, H.; Leklem, J.; Miller, L. T. Measurement of glycosylated vitamin B_6 in foods. *J. Food Sci.* 1983a, *48*, 1422–1425.

Kabir, H.; Leklem, J.; Miller, L. T. Relationship of the glycosylated vitamin B_6 content of foods to vitamin B_6 bioavailability in humans. *Nutr. Rep. Int.* 1983b, *28*, 709–716.

Navankasattusas, S.; Lund, D. B. Thermal destruction of vitamin B_6 vitamers in buffer solutions and cauliflower puree. *J. Food Sci.* 1982, *47*, 1512–1518.

Nguyen, L. B.; Gregory III, J. F. Effects of food composition on the bioavailability of vitamin B-6 in the rat. *J. Nutr.* 1983, *113*, 1550–1560.

Orr, M. L. *Pantothenic acid, vitamin B-6, and vitamin B-12 in Foods*; Home Economics Research Report 36; U.S. Department of Agriculture, U.S. Government Printing Office: Washington, DC, 1969.

Polyanovskii, O. L. The synthesis and some properties of epsilon-N-pyridoxyl lysine. *Biokhimiya* 1963, *28*, 903–906.

Posati, L. P.; Holsinger, V. H.; DeVlbiss, E. D.; Pallansch, M. J. Factors affecting the determination of available lysine in whey with 2,4,6-trinitrobenzenesulfonic acid. *J. Dairy Sci.* 1972, *55*, 1660–1665.

SAS Institute. *Statistical Analysis System*; SAS Institute: Cary, NC, 1985.

Schroeder, H. A. Losses of vitamins and trace minerals resulting from processing and preservation of foods. *Am. J. Clin. Nutr.* 1971, *24*, 562–573.

Severin, E. S.; Gulyaev, N. N.; Khurs, E. N.; Khomutov, R. M. The synthesis and properties of phosphopyridoxyl amino acids. *Biochem. Biophys. Res. Commun.* 1969, *35*, 318–323.

Snyderman, S. E.; Holt, L. E.; Carretero, R.; Jacobs, K. Pyridoxine deficiency in the human infant. *Am. J. Clin. Nutr.* 1953, *1*, 200–207.

Souci, S. W.; Fachmann, W.; Kraut, H. *Food Composition and Nutrition Tables 1986/87*; Deutsche Forschungsanstalt für Lebensmittelchemie, Wissenschaftliche Verlagsgesellschaft mbH: Stuttgart, 1986.

Srncova, V.; Davidek, J. Reaction of pyridoxal and pyridoxal-5-phosphate with proteins. *J. Food Sci.* 1972, *37*, 310–312.

Tarr, J. B.; Tamura, T.; Stokstad, E. L. F. Availability of vitamin B_6 and pantothenate in an average American diet in man. *Am. J. Clin. Nutr.* 1981, *34*, 1328–1337.

Tomarelli, R. M.; Spence, E. R.; Bernhart, F. W. Biological availability of vitamin B-6 of heated milk. *J. Agric. Food Chem.* 1955, *3*, 338–341.