

Total and Reactive Lysine Contents in Selected Cereal-Based Food Products

Nour Mohammad Torbatinejad, † Shane M. Rutherfurd, *,‡ and Paul J. Moughan $^{\$}$

Department of Animal Science, Gorgan University of Agricultural Sciences & Natural Resources, Iran, Institute of Food Nutrition and Human Health, Massey University, Palmerston North, New Zealand, and Riddet Centre, Massey University, Palmerston North, New Zealand

The aim of the study was to determine and compare reactive and total lysine contents in a range of breakfast cereal products. Crude fiber, fat, ash, and crude protein contents of 20 breakfast cereal products ranged from 4 to 38, 14 to 144, 7 to 32, and 52 to 253 g/kg, respectively. The concentrations of glutamic acid (18.7–32.1 g/100 g protein) and proline (4.7–10.8 g/100 g protein) were high while those of the amino acids methionine (1.2–2.0 g/100 g protein) and histidine (1.2–3.3 g/100 g protein) were relatively low. There was a strong relationship between reactive lysine determined using the guanidination and fluorodinitrobenzene methods (R = 0.99). The total lysine content, determined after conventional acid hydrolysis, ranged from 0.8 to 3.7 g/100 g protein, while the reactive lysine content (guanidination) ranged from 0.4 to 2.8 g/100 g protein. Reactive lysine was 20–54% lower than total lysine in the cereal products. The large differences between total and reactive lysine suggest a considerable loss of lysine in the breakfast cereals tested.

KEYWORDS: Lysine; reactive lysine; food; cereals; processing

INTRODUCTION

Cereals have been an important agricultural crop for thousands of years because of their ready cultivation, their reliability as a source of food, and their ease of processing (1). Moreover, cereals are an important source of dietary protein for humans. Relative to other protein sources, however, cereals contain low amounts of lysine, and consequently, lysine is often the first limiting amino acid in diets that are high in cereals. Lysine is also easily modified chemically during processing (2), and this damage may be exacerbated in breakfast cereal products, which often contain added sugars. Ready-to-eat cereals based on maize, wheat, rice, or oats tend to have low protein digestibilities (3) probably largely due to the processing involved in their preparation. Erbersdobler and Hupe (4) found that some 20% of lysine was inactivated and 10% was destroyed in a processed breakfast cereal.

In foodstuffs that have undergone processing or prolonged storage, the ϵ -amino group of lysine can react with other compounds, particularly reducing sugars, rendering the lysine nutritionally unavailable (2, 5, 6). In such processed foods, conventional amino acid analysis, involving acid hydrolysis, overestimates the lysine content due to the breakdown of damaged lysine derivatives present in the food. Because of this, alternative chemical analysis methods have been developed to allow an accurate determination of the amounts of chemically reactive lysine present in foods (2).

There are several chemical methods that can be used to determine the amount of reactive lysine present in processed and stored foods. Arguably the most commonly used methods for determining reactive lysine are the guanidination (7-10) and fluorodinitrobenzene (FDNB) (11) methods.

The aim of the present study was to apply the guanidination method as well as conventional lysine analysis (acid hydrolysis) to a range of commercially available cereal products to determine the extent of lysine damage in the products. The FDNB method was also applied to a subset of the cereal products to demonstrate that the two methods gave similar results.

MATERIALS AND METHODS

Cereal Samples. Twenty commercially available packaged breakfast cereal products were identified. For each cereal product, six different packets, each representing a different processing batch, were purchased from supermarkets in Palmerston North, New Zealand. The different products were produced from different factories while the different batches were produced at different times from the same factory. One hundred grams of material was randomly sampled from each of the six different batches for each of the 20 breakfast cereals giving 120 samples in total. The samples were ground through a 0.5 mm mesh using a Cyclotec 1093 sample mill (Foss Tecator AB, Hoeganaes, Sweden), individually placed into sealed plastic bags, and stored at -20 °C prior to chemical analysis.

^{*} To whom correspondence should be addressed. Tel: 64 6 350 5894. Fax: 64 6 350 5657. E-mail: S.M.Rutherfurd@massey.ac.nz.

[†] Gorgan University of Agricultural Sciences & Natural Resources.

[‡] Institute of Food Nutrition and Human Health, Massey University.

[§] Riddet Centre, Massey University.

Table 1. Ingredient Composition^a of the Cereal-Based Products

| food no. | physical form | cereal | sweetener/flavors | other |
|----------|--------------------|--------------------------|----------------------------|-----------------------------------|
| 1 | biscuit (shredded) | whole wheat 58% | sugar | vitamins |
| | | wheat bran 24% | honey | minerals |
| | | wheat starch | salt, coconut | |
| 2 | biscuit (shredded) | whole wheat 96% | sugar, salt, malt | vitamins |
| 3 | biscuit (shredded) | whole wheat 77% | sugar, fruit 5%, dextrose, | vitamins, minerals, antioxidants, |
| | | | glucose, malt | humectant |
| 4 | biscuit (shredded) | whole wheat 74% | fruit 25%, oil | vitamins |
| 5 | biscuit (shredded) | wheat 97% | sugar, malt | |
| 6 | flaked | corn 60% | sugar, oil, malt, | minerals |
| | | | cocoa 4.5% | |
| 7 | flaked | corn 48% | sugar, skim milk, | |
| | | | cocoa 2.5% | |
| 8 | flaked | cereal 44% (wheat and | sugar, malt | vitamins, minerals, |
| | | maize flour, oat meal) | | sodium bicarbonate |
| 9 | flaked | cereal 62% (whole wheat, | sugar, sultanas 28%, | vitamins, minerals, |
| | | wheat bran) | malt, gluten | humectant |
| 10 | flaked | whole wheat 68%, | sugar, malt | vitamins, minerals |
| | | wheat bran 20% | | |
| 11 | puffed | whole rice 54% | sugar, cocoa, dextrose, | vitamins, minerals |
| | | | malt, gluten, | |
| | | | milk powder | |
| 12 | puffed | rice 98% | sugar, salt | vitamins, minerals |
| 13 | puffed | rice, wheat, corn | sugar, honey 11.2%, | acidity regulator |
| | | | salt | |
| 14 | puffed | cereal 44% (wheat, rice, | sugar, chocolate, | |
| | | oat meal) | milk powder | |
| 15 | puffed | whole wheat 100% | | |
| 16 | rolled | oat 60%, oat bran 20%, | sugar, honey, fruit, | |
| | | wheat flake | glucose, | |
| | | | cinnamon | |
| 17 | rolled | oat rolled 100% | | |
| 18 | rolled | oat rolled 99.8% | salt | |
| 19 | rolled | oat rolled | sugar, honey, fruit, | |
| | | | golden syrup, | |
| | | | salt, flavor | |
| 20 | rolled | cereals 67% (rolled oat, | sugar, fruit 24%, salt, | acidity regulator, |
| | | wheat, corn flour) | flavor, | humectant |
| | | . , | milk powder | |
| | | | | |

^a Summarized from the statutory labeling information given on the product.

Chemical Analysis. Dry matter, ash, crude protein, crude fiber, and total fat contents of the breakfast cereals were determined on a composite sample consisting of equal amounts of material from each batch for each breakfast cereal. FDNB reactive lysine was determined on one randomly selected batch for each cereal product. Gross amino acids (including total lysine) and reactive lysine (guanidination) were determined on all six batches for each of the 20 products.

Dry matter, ash, crude protein, crude fiber, and total fat were determined according to the methods described by AOAC (12). Nitrogen free extractive (NFE) was determined as the difference between the total sample weight and the sum of the moisture, ash, crude protein, crude fiber, and ether extract. FDNB reactive lysine was determined according to the method of Carpenter (11) using the modifications described by Booth (13). Samples containing approximately 10 mg of reactive lysine (estimated previously using amino acid analysis) were reacted with FDNB in ethanol/NaHCO₃ at room temperature for 2 h. The resulting DNP-lysine was liberated from the protein by hydrolysis in 8.1 M HCL for 16 h under reflux conditions. The unreacted FDNB was removed by diethyl ether extraction, and the remaining DNP-lysine was detected by absorbance at 435 nm.

The amino acid content of each sample, including total lysine content, was determined in duplicate using a Waters ion exchange highperformance liquid chromatography system, utilizing postcolumn ninhydrin detection (proline was determined from the absorbance at 440 nm while the remaining amino acids were determined from the absorbance at 570 nm), following hydrolysis in 6 M glass-distilled HCl containing 0.1% phenol for 24 h at 110 \pm 2 °C in evacuated sealed tubes. Tryptophan and cysteine were not determined. The weight of each amino acid was calculated using the free amino acid molecular weight (14).

Reactive lysine was determined using the guanidination method as described by Rutherfurd and Moughan (5). The samples were incubated for 7 days in 0.6 M O-methylisourea (pH 10.6) at 21 ± 2 °C in a shaking water bath, with the reagent-to-lysine ratio greater than 1000, before being dried down and analyzed as described for the amino acid analysis.

Data Analysis. Amino acid (including reactive lysine) contents were compared across cereals using analysis of variance (15). Total (conventional amino acid analysis) and reactive (guanidination) lysine contents were compared, within each product, using a paired *t*-test (15). Correlation analysis was undertaken to examine the degree of relationship between reactive lysine as determined by the guanidination and FDNB methods (15).

RESULTS

The major ingredients for each cereal product, as given on the statutory package label, are presented in **Table 1**. The major constituents differed among the products but were limited to one or more of the following ingredients: corn, wheat, rice, and oats.

The determined proximate composition for each cereal product is presented in **Table 2**. The crude protein ranged from 52 to 253, crude fiber from 4 to 38, total fat from 14 to 144, and ash from 7 to 32 g/kg. The NFE ranged from 678 to 908

Table 2. Mean (±SEM) Nutrient Compositions (g/kg Dry Matter)^{*a*} for the 20 Cereal-Based Products

| cereal product | DM ^b (g/kg air-dry weight) | ash | crude protein | NFE ^c | crude fiber | total fat |
|-------------------|---|----------------|------------------|------------------|----------------|-----------------|
| 1 | 915 ± 0.1 | 29.9 ± 0.1 | 124 ± 0.5 | 746 ± 3.4 | 37.9 ± 1.4 | 61.9 ± 1.4 |
| 2 | 915 ± 0.1 | 19.7 ± 0.0 | 131 ± 0.0 | 807 ± 1.1 | 19.3 ± 0.7 | 22.9 ± 0.4 |
| 3 | 889 ± 0.4 | 29.8 ± 0.3 | 92 ± 3.3 | 834 ± 6.5 | 24.6 ± 2.4 | 19.8 ± 0.5 |
| 4 | 909 ± 0.8 | 15.6 ± 0.4 | 103 ± 1.7 | 833 ± 4.5 | 23.6 ± 1.1 | 24.4 ± 1.3 |
| 5 | 915 ± 0.1 | 21.9 ± 0.1 | 131 ± 0.2 | 790 ± 1.3 | 23.0 ± 0.2 | 34.1 ± 0.8 |
| 6 | 936 ± 0.2 | 22.4 ± 0.1 | 63 ± 1.0 | 875 ± 3.7 | 5.1 ± 0.8 | 34.3 ± 1.8 |
| 7 | 942 ± 0.2 | 20.6 ± 0.4 | 53 ± 0.0 | 908 ± 1.3 | 3.9 ± 0.1 | 14.6 ± 0.8 |
| 8 | 937 ± 0.1 | 25.6 ± 0.2 | 253 ± 2.0 | 679 ± 3.2 | 5.4 ± 0.9 | 36.8 ± 0.1 |
| 9 | 906 ± 0.4 | 30.4 ± 0.2 | 101 ± 0.1 | 793 ± 2.7 | 35.6 ± 0.3 | 40.2 ± 2.1 |
| 10 | 932 ± 0.1 | 32.4 ± 0.1 | 127 ± 0.4 | 783 ± 5.4 | 23.0 ± 0.3 | 34.9 ± 4.6 |
| 11 | 941 ± 0.1 | 24.4 ± 0.0 | 52 ± 1.0 | 902 ± 1.6 | 3.7 ± 0.1 | 18.1 ± 0.5 |
| 12 | 925 ± 0.1 | 26.8 ± 0.1 | 67 ± 0.6 | 889 ± 1.9 | 3.7 ± 0.7 | 13.9 ± 0.5 |
| 13 | 917 ± 0.2 | 7.4 ± 0.3 | 54 ± 1.5 | 904 ± 5.7 | 6.6 ± 0.1 | 28.1 ± 3.8 |
| 14 | 933 ± 0.1 | 24.5 ± 0.5 | 91 ± 0.3 | 826 ± 2.4 | 6.2 ± 0.7 | 52.2 ± 0.9 |
| 15 | 906 ± 0.6 | 14.2 ± 0.1 | 132 ± 0.1 | 783 ± 2.4 | 17.8 ± 0.9 | 53.3 ± 1.3 |
| 16 | 911 ± 0.6 | 20.1 ± 0.7 | 121 ± 2.5 | 678 ± 9.0 | 36.0 ± 0.5 | 144.7 ± 5.3 |
| 17 | 931 ± 0.1 | 16.5 ± 0.4 | 146 ± 0.4 | 707 ± 2.5 | 23.0 ± 0.2 | 108 ± 1.5 |
| 18 | 921 ± 0.0 | 22.2 ± 0.5 | 146 ± 0.4 | 718 ± 2.9 | 20.8 ± 0.3 | 93.2 ± 1.7 |
| 19 | 916 ± 0.7 | 17.2 ± 0.2 | 124 ± 0.3 | 699 ± 2.8 | 21.0 ± 0.4 | 138.8 ± 1.9 |
| 20 | 920 ± 0.4 | 15.7 ± 0.0 | 124 ± 0.0 | 734 ± 0.7 | 19.1 ± 0.1 | 107.2 ± 0.6 |

^a Mean values based on duplicate determinations on a composite sample consisting of equal amounts from each of six batches for each of the 20 breakfast samples. ^b DM = dry matter. ^c NFE = nitrogen-free extractive.



Figure 1. Plot of values for reactive lysine in the 20 cereal-based food products determined by either the FDNB or the guanidination (O-methylisourea) methods. The solid line shown denotes complete agreement between the two methods.

g/kg and demonstrated, as expected, that carbohydrates were the main chemical components of the products.

FDNB reactive lysine contents determined on a randomly selected single batch for each breakfast cereal were compared to reactive lysine contents (for the same batch of cereal) determined using the guanidination method. The reactive lysine content (guanidination) was plotted against reactive lysine (FDNB) (**Figure 1**). The correlation coefficient (r) was 0.985 (p < 0.001) indicating a high degree of correlation between the two methods.

Amino acid contents were determined for all six batches for each of the 20 breakfast cereals (**Table 3**). Overall, the mean (n = 6 batches) amino acid contents for the 20 cereals were significantly different (p < 0.001) between the cereal products for each amino acid.

The coefficient of variation (CV) was calculated for each amino acid across the six batches for each breakfast cereal, and the mean CV for all of the amino acids within each food was calculated. The resultant measure of interbatch variability for each cereal is shown in **Table 4**. The CV values for the determined amino acid contents ranged from 5.6 to 48% indicating a high degree of variation for amino acid contents between batches within a cereal product.

The total and reactive (guanidination) lysine contents are shown in Table 5. The total lysine content for the breakfast cereals ranged from 0.8 g/100 g protein in a corn-based cereal (cereal 7) to 3.7 g/100 g protein in an oat-based cereal (cereal 19) with an overall mean of 2.2 g/100 g protein for all of the 20 cereals. The reactive lysine content ranged from 0.4 g/100g protein in cereal 8 to 2.8 g/100 g protein in cereal 18 with an overall mean of 1.4 g/100 g protein for all 20 cereals. In general, the cereals based on corn contained the lower amounts of reactive lysine (0.6-0.9 g/100 g protein for cereals 6, 7, and 13). The cereals that contained rice had reactive lysine contents ranging from 1.2 to 1.3 g/100 g protein (cereals 11 and 12), and those that contained wheat ranged from 0.4 to 1.7 g/100 g protein (cereals 1-5, 8-10, 14, and 15). The cereals that contained oats had the highest amount of reactive lysine (1.9-2.8 g/100 g protein for cereals 16-20). For all breakfast cereals, the total lysine was significantly (p < 0.001) higher than the reactive lysine. The difference between the total lysine and the reactive lysine gives an estimate of the minimum amount of modified lysine present in the breakfast cereals. Essentially, when the modified lysine derivatives undergo acid hydrolysis, some but not all revert back to lysine. Therefore, the difference between the total lysine and the reactive lysine values reflects only a portion of the modified lysine present. Modified lysine, estimated in this way, although being a minimum value, does highlight the extensive lysine modification that has occurred in these cereals. The difference between the total lysine and the reactive lysine was calculated as a percentage of reactive lysine and is shown in Table 5. Differences ranged from 19 (cereal 18) to 54% (cereal 9) with a mean of 38%.

DISCUSSION

Reactive lysine is a more accurate measure of unmodified lysine present in a processed foodstuff than total lysine since total lysine determinations often include lysine that has reverted from modified lysine derivatives during acid hydrolysis. There are a number of methods for measuring reactive lysine in foodstuffs, two of which are the guanidination method and the FDNB method. The aim of the present study was to apply the latter two methods to determine total and reactive lysine contents for a range of breakfast cereals.

The guanidination method has been validated in several studies (7-10) and is a suitable method for determining reactive lysine in foods. The FDNB method, however, is perhaps more commonly used despite being slow and laborious to perform. The correlation between the two methods was high (r = 0.99; regression equation, y = 1.05x + 0.01), and it would appear that both methods can be used to determine reactive lysine in breakfast cereals with confidence. The mean CV for the guanidination method when applied to the 20 cereals was 2.6% while for the FDNB method it was 3.6%. Overall, the precision of the guanidination method was better than that for the FDNB assay.

Total lysine significantly (p < 0.001) overestimated reactive lysine in all of the cereals tested. This is typical of protein sources that have been subjected to processing or prolonged storage where lysine has undergone Maillard type reactions (14). The calculation of lysine damage reflects the minimal possible

Table 3. Mean^a Amino Acid Composition^b (g/100 g Protein^c) for the 20 Cereal Products

| | | | | | | | | | | | | | | | | | | | | | | overall |
|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|--------|---------------------------|
| cereal | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | SE^d | significance ^e |
| aspartic acid | 5.4 | 4.9 | 5.6 | 5.4 | 5.1 | 6.1 | 6.6 | 3.9 | 6.0 | 4.8 | 10.5 | 8.2 | 6.0 | 6.8 | 5.2 | 7.3 | 7.2 | 7.8 | 8.8 | 6.8 | 0.14 | *** |
| threonine | 2.7 | 2.5 | 2.7 | 2.5 | 2.5 | 2.9 | 3.0 | 2.1 | 2.8 | 2.4 | 3.8 | 2.9 | 2.9 | 2.8 | 2.5 | 2.7 | 2.7 | 2.9 | 3.3 | 2.6 | 0.14 | *** |
| serine | 3.8 | 3.8 | 4.0 | 4.0 | 3.8 | 4.1 | 4.4 | 3.6 | 3.8 | 3.8 | 5.5 | 4.5 | 4.3 | 4.3 | 3.8 | 3.8 | 3.8 | 4.1 | 4.4 | 3.7 | 0.35 | *** |
| glutamic acid | 24.9 | 28.3 | 29.7 | 26.1 | 27.4 | 19.1 | 19.6 | 32.1 | 21.8 | 28.1 | 22.9 | 18.8 | 24.1 | 24.9 | 24.8 | 19.3 | 19.9 | 19.5 | 20.7 | 18.7 | 0.16 | *** |
| proline | 8.4 | 9.1 | 10.1 | 9.1 | 9.0 | 8.1 | 7.9 | 10.8 | 7.8 | 9.1 | 6.0 | 4.7 | 8.1 | 7.7 | 7.9 | 5.1 | 5.2 | 4.9 | 5.2 | 4.7 | 0.23 | *** |
| glycine | 4.1 | 3.8 | 4.2 | 4.0 | 3.7 | 3.4 | 3.1 | 3.2 | 4.4 | 3.9 | 5.3 | 4.1 | 3.8 | 4.1 | 3.8 | 4.5 | 4.2 | 4.7 | 5.2 | 4.1 | 0.23 | *** |
| alanine | 3.9 | 3.5 | 4.1 | 3.7 | 3.5 | 6.3 | 6.9 | 2.9 | 4.4 | 3.4 | 6.3 | 5.4 | 5.4 | 4.0 | 3.6 | 4.3 | 4.1 | 4.6 | 5.2 | 4.1 | 0.26 | *** |
| valine | 4.0 | 3.8 | 4.3 | 4.1 | 3.8 | 4.4 | 4.7 | 3.5 | 4.2 | 3.9 | 6.3 | 5.3 | 4.8 | 4.5 | 4.0 | 4.4 | 4.4 | 4.8 | 5.3 | 4.3 | 0.50 | *** |
| methionine | 1.3 | 1.3 | 1.4 | 1.3 | 1.3 | 1.5 | 1.6 | 1.2 | 1.3 | 1.2 | 2.1 | 2.0 | 1.6 | 1.4 | 1.3 | 1.3 | 1.3 | 1.5 | 1.6 | 1.3 | 0.25 | *** |
| isoleucine | 3.0 | 3.0 | 3.3 | 3.1 | 3.0 | 3.1 | 3.5 | 3.0 | 3.0 | 3.0 | 4.4 | 3.7 | 3.5 | 3.3 | 3.1 | 3.2 | 3.2 | 3.3 | 3.8 | 3.1 | 0.14 | *** |
| leucine | 5.9 | 6.1 | 7.2 | 6.1 | 6.0 | 10.3 | 11.5 | 6.3 | 6.3 | 6.1 | 9.2 | 7.9 | 8.8 | 6.8 | 6.2 | 6.5 | 6.6 | 7.0 | 7.7 | 6.2 | 0.76 | *** |
| tyrosine | 2.7 | 2.8 | 3.3 | 2.8 | 2.6 | 3.6 | 4.3 | 2.9 | 2.8 | 2.8 | 5.2 | 4.8 | 3.8 | 3.3 | 3.0 | 3.1 | 3.2 | 3.5 | 3.6 | 3.0 | 0.26 | *** |
| phenylalanine | 3.9 | 4.0 | 4.4 | 3.9 | 3.9 | 4.4 | 4.9 | 4.2 | 3.7 | 3.9 | 5.7 | 4.8 | 4.7 | 4.4 | 3.9 | 4.2 | 4.4 | 4.6 | 5.0 | 4.2 | 0.29 | *** |
| histidine | 1.5 | 1.8 | 2.4 | 2.2 | 2.0 | 3.0 | 3.3 | 2.2 | 3.0 | 2.5 | 2.6 | 2.2 | 1.2 | 2.0 | 1.7 | 2.1 | 2.1 | 1.9 | 2.5 | 2.0 | 0.44 | *** |
| arginine | 4.2 | 3.5 | 3.6 | 4.5 | 3.3 | 3.7 | 3.1 | 2.7 | 5.0 | 3.5 | 8.1 | 6.4 | 4.2 | 4.9 | 4.0 | 6.4 | 5.6 | 6.4 | 7.7 | 5.7 | 1.18 | *** |

^a Mean of duplicate determinations conducted on each of six batch samples for each product. ^b Performic acid oxidation of methionine was not carried out, and methionine was determined by acid hydrolysis alone after thorough degassing of the sample to remove oxygen. Cysteine and tryptophan were not determined. ^c Protein was calculated as nitrogen multiplied by 5.83. ^d Overall standard error. ^e *** denotes p < 0.001.

| Table 4. Me | ean CV (%) ^a fo | r the Amino Acid | Content (Variation within |
|--------------|----------------------------|-------------------|---------------------------|
| Cereal acros | ss Six Batches |) for Each of the | 20 Breakfast Cereals |

| cereal product | coefficient of variation | cereal product | coefficient of variation |
|----------------|--------------------------|----------------|--------------------------|
| 1 | 6.0 | 11 | 47.7 |
| 2 | 5.9 | 12 | 15.7 |
| 3 | 14.0 | 13 | 26.0 |
| 4 | 7.5 | 14 | 19.5 |
| 5 | 8.4 | 15 | 40.9 |
| 6 | 15.5 | 16 | 26.0 |
| 7 | 26.3 | 17 | 10.4 |
| 8 | 9.4 | 18 | 11.6 |
| 9 | 10.4 | 19 | 19.7 |
| 10 | 5.6 | 20 | 15.6 |

^a A coefficient of variation (CV) (across batches) was calculated for each amino acid in each product. The value shown is the overall mean CV determined across all of the amino acids for each cereal.

damage that the lysine in the original cereal has undergone after processing and storage. The difference between total lysine and reactive lysine reflects reversion of early Maillard products present in the cereals. Because it is likely that there are also late Maillard products present and that these will not revert back to lysine thus contributing to the total lysine, the actual amount of chemically modified lysine is likely to be higher than determined here. Lysine damage was extensive with a minimum of 20-50% of the lysine being modified. There are a number of implications based on the present finding. First, the actual lysine present in the breakfast cereals is much lower than would have been predicted based on the formulation. Second, given that the processing conditions were severe enough to cause the extent of lysine damage observed, it is possible that other amino acids, for example, cysteine and methionine, would also have been modified or destroyed (16), and third, it would appear that there are quantitatively significant amounts of Maillard products present in the breakfast cereals, which would be consumed as part of the breakfast cereal.

With the severity of lysine damage observed in the present study, it is anticipated that the digestibility of undamaged lysine in the cereals may also be adversely affected (17). Consequently, it would be useful to determine ileal digestible reactive (available) lysine (14) in the breakfast cereals to fully quantify available lysine contents. This will be the subject of further study.

| Table 5. Mean ^a Total (Conventional Analysis) and Reactive |
|---|
| (Guanidination) Lysine Contents (g/100 g Protein ^b) for the 20 Cereal |
| Products |

| cereal | ly | vsine | overall | | difference | | |
|---------|-------|----------|---------|---------------------------|------------|----------------|--|
| product | total | reactive | SEM | significance ^c | g/kg | % ^d | |
| 1 | 2.2 | 1.5 | 0.03 | *** | 0.73 | 31 | |
| 2 | 1.8 | 1.1 | 0.04 | *** | 0.69 | 37 | |
| 3 | 2.1 | 1.2 | 0.04 | *** | 0.87 | 45 | |
| 4 | 2.5 | 1.7 | 0.04 | *** | 0.78 | 31 | |
| 5 | 1.7 | 1.2 | 0.04 | *** | 0.46 | 30 | |
| 6 | 1.4 | 0.8 | 0.02 | *** | 0.63 | 44 | |
| 7 | 0.9 | 0.6 | 0.01 | *** | 0.38 | 42 | |
| 8 | 0.8 | 0.4 | 0.04 | *** | 0.36 | 44 | |
| 9 | 2.2 | 1.0 | 0.03 | *** | 1.19 | 54 | |
| 10 | 1.6 | 0.9 | 0.04 | *** | 0.71 | 42 | |
| 11 | 2.5 | 1.3 | 0.02 | *** | 1.15 | 45 | |
| 12 | 1.9 | 1.2 | 0.03 | *** | 0.75 | 43 | |
| 13 | 1.5 | 0.9 | 0.02 | *** | 0.56 | 38 | |
| 14 | 2.4 | 1.2 | 0.06 | *** | 1.21 | 47 | |
| 15 | 1.2 | 0.7 | 0.02 | *** | 0.53 | 43 | |
| 16 | 3.6 | 2.5 | 0.08 | *** | 1.16 | 31 | |
| 17 | 3.4 | 2.5 | 0.05 | *** | 0.89 | 27 | |
| 18 | 3.5 | 2.8 | 0.06 | *** | 0.68 | 19 | |
| 19 | 3.7 | 2.4 | 0.08 | *** | 1.29 | 34 | |
| 20 | 3.2 | 1.9 | 0.06 | *** | 1.29 | 41 | |

^{*a*} Mean based on duplicate determinations conducted across six batch samples for each of the 20 breakfast samples. ^{*b*} Protein was calculated as nitrogen multiplied by 5.83. ^{*c*} *** denotes p < 0.001. ^{*d*} % difference was calculated as follows: (total lysine – reactive lysine) × 100/reactive lysine.

There are a number of factors that are likely to affect the extent of the lysine damage in the breakfast cereals. These include cereal grain composition, storage conditions of the raw material, physical characteristics of the primary process, flavor additives and fortifying ingredients, as well as the manufacturing process and storage conditions of the finished products (2, 5, 18, 19). Because the specific processing conditions of the 20 breakfast cereals tested in this study were not known, any conclusions drawn about the influence of processing on lysine damage would be speculative.

The amino acid compositions of the 20 breakfast cereals were significantly different between cereals. This is to be expected given the different protein contents of the ingredients and the range of protein ingredients used. The amino acid profile tended to reflect the profile of the major ingredients. Wheat, for example, has a high amount of glutamic acid and proline, and this was reflected in the wheat-based cereals (1-5, 8-10, 13-15), which also had high levels of glutamic acid and proline. Similarly with corn, the corn-based cereals had high levels of glutamic acid, leucine, and aspartic acid, while the rice-based and oats-based cereals had high levels of glutamic acid, aspartic acid, and arginine as do rice and oats. Corn-based cereals were low in methionine (methionine was determined without performic acid oxidation prior to hydrolysis), threonine, and glycine, as is corn. Moreover, wheat-based, rice-based, and oat-based cereals were low in methionine, threonine, and histidine, which again reflected the cereal bases used. When the essential amino acid composition was compared to the requirements for children (10-12 years) (20), lysine was the most limiting amino acid in all cereals with the exception of cereals 16–18 for which methionine/cysteine were the most limiting.

In conclusion, the crude protein and total lysine contents of the tested breakfast cereals varied over a wide range. High proportions of glutamic acid, aspartic acid, and proline and low proportions of methionine, threonine, and histidine were characteristic of the breakfast cereal products, and lysine appeared to be the most limiting amino acid. For all of the cereal products, total lysine contents were considerably greater than reactive lysine contents indicating that there was a significant degree of lysine damage. Because total lysine values are used in practice for describing food lysine contents, the protein quality of the breakfast cereals is considerably poorer than is currently recognized.

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