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# Effect of Heat Damage in an Autoclave on the Reactive Lysine Contents of Soy Products and Corn Distillers Dried Grains with Solubles. Use of the Results To Check on Lysine Damage in Common Qualities of These Ingredients

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The suitability of the homoarginine reaction for determining the reactive lysine in soy products and corn distillers dried grain with solubles (DDGS) was tested. For this purpose, some batches were subjected to deliberate heat damage for up to 30 min in an autoclave with 135 °C hot steam, and the samples were analyzed for total lysine and reactive lysine. In addition, 84 samples of common soy and 80 samples of corn DDGS were tested for their content of total and reactive lysine, and the contents were compared with those of the autoclave tests. For soy products conclusive results were obtained. In the case of heat treatment, both total lysine and reactive lysine decrease, but the latter is clearly a more sensitive indicator of lysine damage. Most normal products are quite similar, with toasting-induced damage to reactive lysine of ca. 15% compared to untoasted beans. The cause of the constantly occurring residual lysine after guanidination and the poorer reaction balance in the case of damage were explained. For common DDGS samples, however, less favorable results were obtained. Reactive and total lysine decreased almost in parallel due to heat damage, showing a great gap between them. Results showed indeed that variation of total and reactive lysine in DDGS is high, proving that its production conditions are not yet optimal for a feed ingredient.

**KEYWORDS: In vitro lysine availability; reactive lysine; total lysine; heat damage; amino acids; homoarginine; guanidination; Maillard reactions**

## INTRODUCTION

The formulation of compound feeds from raw materials based on amino acid requirements is practiced worldwide today, and the essential amino acid lysine is often limiting to growth and must be supplemented. In feedstuffs that have undergone processing or even prolonged storage, the $\epsilon$-amino group of protein-bound lysine can react with other compounds to render the amino acid unavailable for nutrition (*1*). Some of these adducts can be broken down further with acid–protein hydrolysis to release lysine. Thus, total lysine content is somewhat uncertain for the formulation of complete feeds. Faldet et al. (*2*) derivatized undamaged reactive lysine by reaction with 1-fluoro-2,4-dinitrobenzene (FDNB) and used the results to conduct research on the optimum toasting of soybeans. Analysis was based on a specific difference method, wherein the chromatographically analyzed residual lysine content after derivatization was subtracted from total lysine. Moughan and Rutherfurd (*3*) compared the guanidination at the $\epsilon$-amino group of lysine to homoarginine, developed by Mauron and Bujard (*4*) with the FDNB reaction. Rutherfurd et al. (*5*) tested, in a comparison with various methods, the determination of reactive lysine in soy products and other raw materials, optimized the reaction conditions, and showed that the guanidination reaction is most suitable for feed ingredients. The waste product of bioethanol manufacture, corn distillers dried grains with solubles (DDGS), which has been produced in increasingly large amounts in recent years, has been introduced as a feedstuff raw material. Fastinger et al. (*6*, *7*) characterized five different DDGS grades according to their color and investigated ileal amino acid digestibility in pigs and amino acid availability in roosters. They observed a particularly great variation with lysine and the lowest digestibilities with dark samples. They interpreted the findings as the products' being induced by the Maillard reaction in the

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manufacture of DDGS. Similar findings, that is, major variation in ileal digestibility of lysine, were also observed by Stein et al. (*8*) with 10 DDGS batches from different ethanol plants, and they concluded also that damage during the manufacturing process was caused by sugar due to incomplete fermentation and starch removal.

The goal of the present study is to test the suitability of the homoarginine reaction in detail for determining reactive (available) lysine in soy products and DDGS and if this value is a more sensitive and realistic damage indicator than the total lysine content normally analyzed. For this purpose, we need both to induce deliberate lysine damage with heat stress in an autoclave and to compare the test results obtained in such a way with the reactive lysine findings in a large number of normal ingredient samples.

## MATERIALS AND METHODS

**Samples.** Eighty-four samples of soybean meals and soybeans and 80 samples of the new feed raw material DDGS, which is produced from corn in bioethanol manufacture in the United States, were obtained over a period of 3 years from feed manufacturers from around the world. Two samples of soybean meal, one full-fat soy, and two DDGS were ordered in 3 kg amounts for heat damage tests. All samples were of feed grade quality. They were ground, using a 0.5 mm sieve, and filled in sealed 250 mL polyethylene bottles. The ground samples were used for all analyses.

**Targeted Heat Damage in the Autoclave.** The tests were conducted at the Deutsches Institut für Lebensmitteltechnik e.V., Quakenbrücken, Germany, using a Certoclav Sterilizer. Tested first were Argentine soybean meals 44/7 and HP, as well as whole-fat soybeans, which were acquired in a large sample quantity. Kilogram amounts were first ground to smaller than 3 mm, homogenized well, and used for the tests. Two hundred and fifty grams of each sample was wrapped in a cloth towel and placed with a basket into the steam phase of boiling water. After the autoclave was sealed, the desired temperature of ca. 135 °C was reached after ca. 3 min. From that time on, heat treatment was measured using different fillings between 3 and 30 min, with 3 min intervals, so that 10 different damaged portions were obtained. An increasing brown coloration of the material was clearly observed, particularly in the fat-containing grade. After each of the times had elapsed, the autoclave was quickly opened and the filled towel was removed. The product was then quick-cooled by spreading it on trays and left to dry overnight on these trays.

In a second test series, two samples of normal DDGS with 24 and 27% crude protein were used. Only time intervals of 5 min were applied so that there were six samples each that were heated between 5 and 30 min. The samples were removed, cooled, and dried like the soy samples.

All samples were sent to our laboratory for analysis of reactive lysine and total amino acid contents.

**Homoarginine Reaction for Determining Reactive Lysine.** *o*-Methylisourea (OMIU) can guanidinize undamaged protein-bound lysine to homoarginine in an alkaline medium (*3*, *4*). The latter is determined chromatographically with an amino acid analyzer after acid–protein hydrolysis and its content converted mathematically to reactive lysine content by multiplication with the molar weight ratio of 0.7767.

OMIU was purchased from Fluka (code 67714) and the homoarginine standard material from Bachem (code F-2780). Before calibration, the standard was chromatographically tested on purity and for foreign amino acids (Lys, Val, etc.) contents. It was pure with no other amino acids (<0.1% detection limit). The standard homoarginine contains crystal water. The Bachem declared content of 66.4% was checked by nitrogen determination applying method 990.03 of the AOAC International (*10*) and used for the calibration.

**Conditions for Optimum Derivatization Yield.** For each feed raw material, the optimum derivatization conditions for transformation of lysine to homoarginine were determined in preliminary trials by varying the pH and reaction time as proposed by Maga (*9*). He has found that an OMIU concentration of 0.6 mol/L is sufficient and that the reaction should be at room temperature. The following conditions were optimal for soy products: pH 11.5; reaction time, 2 days. For DDGS the optimal conditions were pH 12 and a reaction time of 2.5 days.

**Derivatization Reagent: OMIU Solution, $c = 0.6$ mol/L.** OMIU (10.3 g) was dissolved in 50 mL of water and adjusted to the optimum pH determined from the raw material with ca. 16 mL of 7.5 mol/L sodium hydroxide solution. After cooling to room temperature, the container was filled to the mark (100 mL) and the pH checked again. The solution was prepared fresh every week and the pH adjusted before use.

**Procedure of Derivatization, Hydrolysis, and Homoarginine Determination.** Ca. 400 mg of soy product or 700 mg of DDGS, respectively—weighed to within 0.1 mg—are combined with 15 mL of OMIU solution in a threaded 100 mL thick-wall laboratory flask constructed of DURAN Glass (Schott) and stirred for the required time at room temperature. Twenty-five milliliters of 9 mol/L hydrochloric acid containing 1 g of phenol per liter is then added to the derivatized sample. Care must be taken that all sample residues adhering to the sidewalls of the vessel are washed off. The hydrolysis procedure followed the conditions of the European Union official analysis of amino acids in feed (*10*). The flask with the prepared mixture is placed in a heating cabinet thermostated to 110 °C. To avoid a pressure rise caused by included air, the threaded lid (red PBTP caps with silicone/Teflon seal) is placed only loosely on the flask for the first hour. After heating for 1 h, the container is sealed tightly and left for 23 h at 110 °C for protein hydrolysis. Subsequently, further preparation is conducted similarly to the analysis for total lysine (see below after the addition of norleucine), and chromatography is performed using the amino acid analyzer. Homoarginine elutes as the last peak ca. 10 min after arginine.

The repeatability of the assay was tested using double determination data of eight soybean meal and eight DDGS samples. For soybean meal the standard deviation of the reactive lysine contents was 0.0152% and that for the small contents of residual lysine, 0.0128%. This is equivalent to a relative error CVr of 0.7% for reactive lysine and 4.9% for residual lysine. For the total lysine determination we also found a CVr of 0.7%. In the case of DDGS the standard deviation of the reactive lysine determination was 0.009% and that for residual lysine, 0.005%. Due to the lower total lysine contents in DDGS the relative error CVr was 1.5% for reactive lysine and 4.8% for residual lysine. Thus, the method is highly precise, and therefore we did normally not apply the homoarginine reaction in duplicate.

**Chemical and Chromatographic Methods.** The nitrogen content of the samples was determined by the Dumas method according to the official method 990.03 of the AOAC International (*10*). Crude protein was obtained using the conversion factor of 6.25. Dry matter (moisture) was determined by drying samples in a ventilated oven for 4 h at 103 °C and weighing them back. Total lysine and other amino acids were analyzed according to a procedure that meets the requirements of the official European method of amino acid analysis in feed (*11*) and of the official method code 994.12 of the AOAC International (*10*). A sample amount containing approximately 10 mg of nitrogen was weighed in; 5 mL of performic acid was added to oxidize methionine to methionine sulfone and cystine to cysteic acid for 16 h in an ice bath. According to the above-mentioned official methods, this step does not interfere with the determination of the other amino acids. After the performic acid was destroyed by adding 0.84 g of sodium metabisulfate, the protein was hydrolyzed for 24 h at 110 °C in a closed 50 mL glass bottle with a screw cap. Then norleucine was added as an internal standard, and the hydrolysate was diluted with buffer and adjusted to a pH of 2.20. The amino acids were separated on a cation exchanger resin and were postcolumn reacted with ninhydrin following photometric detection at 570 nm. For the detailed wet chemical procedure see Oxidation and Hydrolysis in ref *12* of Degussa AG and Llames and Fontaine (*13*).

## RESULTS AND DISCUSSION

**Conditions of the Guanidination Reaction.** The reaction conditions used in our laboratory for guanidination of the protein-bound lysine differ somewhat from those described in ref *3*. The optimal pH of the OMIU is directly adjusted by

**Table 1.** Effect of Heat Treatment of Soy Products in a Steamed Autoclave at 135 °C on the Content of Total and Reactive Lysine

| sample | heat treatment | total Lys | reactive Lys | residual Lys | sum of rLys and Lys-res | total Lys/CP | loss of total Lys/CP (%) | reactive Lys/CP | loss of reactive Lys/CP (%) |
|---|---|---|---|---|---|---|---|---|---|
| soybean meal HP, CP = 46.8% | 0 | 2.89 | 2.39 | 0.23 | 2.62 | 6.18 | 0.0 | 5.1 | 0.0 |
| | 3 | 2.85 | 2.35 | 0.27 | 2.62 | 6.09 | 1.4 | 5.02 | 1.7 |
| | 6 | 2.81 | 2.26 | 0.27 | 2.53 | 6.00 | 2.8 | 4.83 | 5.4 |
| | 9 | 2.72 | 2.13 | 0.31 | 2.44 | 5.81 | 5.9 | 4.55 | 10.9 |
| | 12 | 2.71 | 2.12 | 0.31 | 2.43 | 5.79 | 6.2 | 4.53 | 11.3 |
| | 15 | 2.63 | 2.00 | 0.37 | 2.37 | 5.62 | 9.0 | 4.27 | 16.3 |
| | 18 | 2.59 | 1.81 | 0.43 | 2.24 | 5.53 | 10.4 | 3.87 | 24.3 |
| | 21 | 2.49 | 1.78 | 0.45 | 2.23 | 5.32 | 13.8 | 3.80 | 25.5 |
| | 24 | 2.45 | 1.65 | 0.5 | 2.15 | 5.24 | 15.2 | 3.53 | 31.0 |
| | 27 | 2.38 | 1.59 | 0.52 | 2.11 | 5.09 | 17.6 | 3.40 | 33.5 |
| | 30 | 2.28 | 1.44 | 0.56 | 2 | 4.87 | 21.1 | 3.08 | 39.7 |
| | slope | *−0.020* | *−0.032* | *0.011* | *−0.021* | *−0.043* | | *−0.069* | |
| | intercept | *2.92* | *2.44* | *0.21* | *2.65* | *6.23* | | *5.21* | |
| | RSQ | *0.987* | *0.987* | *0.976* | *0.984* | *0.987* | | *0.987* | |
| soybean meal 44/7, CP = 42.6% | 0 | 2.67 | 2.2 | 0.21 | 2.41 | 6.27 | 0.0 | 5.16 | 0.00 |
| | 3 | 2.6 | 2.1 | 0.23 | 2.33 | 6.10 | 2.6 | 4.93 | 4.55 |
| | 6 | 2.49 | 1.96 | 0.27 | 2.23 | 5.85 | 6.7 | 4.60 | 10.91 |
| | 9 | 2.55 | 2.02 | 0.26 | 2.28 | 5.99 | 4.5 | 4.74 | 8.18 |
| | 12 | 2.42 | 1.83 | 0.32 | 2.15 | 5.68 | 9.4 | 4.30 | 16.82 |
| | 15 | 2.33 | 1.71 | 0.34 | 2.05 | 5.47 | 12.7 | 4.01 | 22.27 |
| | 18 | 2.26 | 1.62 | 0.37 | 1.99 | 5.31 | 15.4 | 3.80 | 26.36 |
| | 21 | 2.26 | 1.57 | 0.39 | 1.96 | 5.31 | 15.4 | 3.69 | 28.64 |
| | 24 | 2.16 | 1.51 | 0.43 | 1.94 | 5.07 | 19.1 | 3.54 | 31.36 |
| | 27 | 2.15 | 1.43 | 0.46 | 1.89 | 5.05 | 19.5 | 3.36 | 35.00 |
| | 30 | 2.05 | 1.29 | 0.47 | 1.76 | 4.81 | 23.2 | 3.03 | 41.36 |
| | slope | *−0.020* | *−0.029* | *0.009* | *−0.020* | *−0.047* | | *−0.069* | |
| | itercept | *2.66* | *2.19* | *0.20* | *2.39* | *6.24* | | *5.14* | |
| | RSQ | *0.971* | *0.980* | *0.987* | *0.968* | *0.971* | | *0.980* | |
| soybeans, full fat, CP = 36.6% | 0 | 2.17 | 1.9 | 0.14 | 2.04 | 5.93 | 0.0 | 5.19 | 0.0 |
| | 3 | 2.17 | 1.82 | 0.17 | 1.99 | 5.93 | 0.0 | 4.97 | 4.2 |
| | 6 | 2.12 | 1.77 | 0.18 | 1.95 | 5.79 | 2.3 | 4.84 | 6.8 |
| | 9 | 2.09 | 1.71 | 0.21 | 1.92 | 5.71 | 3.7 | 4.67 | 10.0 |
| | 12 | 2.04 | 1.61 | 0.24 | 1.85 | 5.57 | 6.0 | 4.40 | 15.3 |
| | 15 | 2.04 | 1.57 | 0.25 | 1.82 | 5.57 | 6.0 | 4.29 | 17.4 |
| | 18 | 2.09 | 1.49 | 0.29 | 1.78 | 5.71 | 3.7 | 4.07 | 21.6 |
| | 21 | 1.94 | 1.34 | 0.33 | 1.67 | 5.30 | 10.6 | 3.66 | 29.5 |
| | 24 | 1.81 | 1.23 | 0.39 | 1.62 | 4.95 | 16.6 | 3.36 | 35.3 |
| | 27 | 1.87 | 1.29 | 0.37 | 1.66 | 5.11 | 13.8 | 3.52 | 32.1 |
| | 30 | 1.81 | 1.24 | 0.4 | 1.64 | 4.95 | 16.6 | 3.39 | 34.7 |
| | slope | *−0.013* | *−0.024* | *0.009* | *−0.015* | *−0.035* | | *−0.065* | |
| | intercept | *2.20* | *1.90* | *0.13* | *2.03* | *6.02* | | *5.20* | |
| | RSQ | *0.872* | *0.967* | *0.973* | *0.955* | *0.872* | | *0.967* | |

adding sodium hydroxide solution, whereas in the method of ref *3* first the OMIU base is produced by adding barium hydroxide under precipitation of barium sulfate and followed by pH adjustment. Additionally, the molar ratio of OMIU to lysine is between 100:1 and 200:1, a definite reagent excess, however, less than the 1000:1 used in the laboratory of Massey University. Our tests showed that, with a 1000-fold excess of OMIU, which cleaves to ammonia in acid hydrolysis, the reaction coil of the amino acid analyzer for postcolumn derivatization can be clogged with reaction products of am-

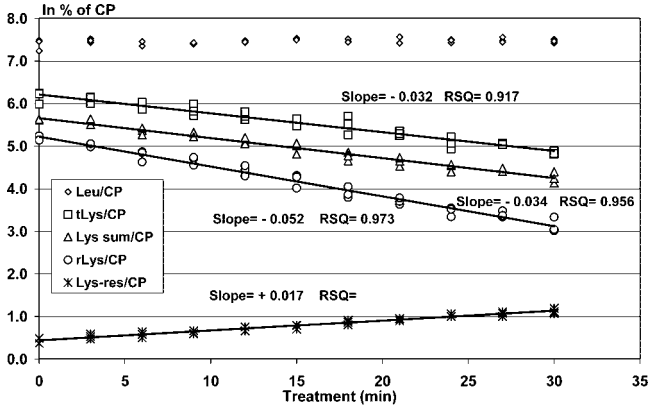


**Figure 1.** Heat treatment of all three soy qualities. Contents relative to crude protein fall together, which shows that the amount of damage is independent of the sample itself.

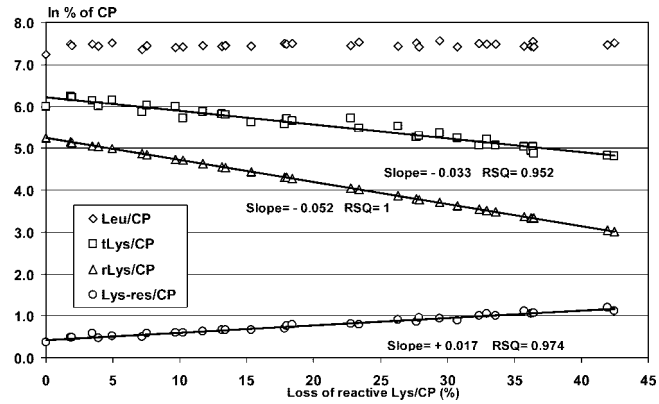


**Figure 2.** Contents of all three soy qualities relative to crude protein dependent on the loss of rLys/CP. Up to 42% of reactive lysine is lost during the heat treatment.

**Table 2.** Effect of Heat Treatment of DDGS in a Steamed Autoclave at 135 °C on the Content of Total and Reactive Lysine

| sample | heat treatment | total Lys | reactive Lys | residual Lys | sum of rLys and Lys-res | total LYS/CP | loss of total Lys/CP (%) | reactive Lys/CP | loss of reactive Lys/CP (%) |
|---|---|---|---|---|---|---|---|---|---|
| distillers dried grains and solubles, CP = 27.0% | 0 | 0.82 | 0.64 | 0.13 | 0.77 | 3.05 | 0.0 | 2.36 | 0.0 |
| | 5 | 0.77 | 0.55 | 0.12 | 0.66 | 2.86 | 6.3 | 2.03 | 14.0 |
| | 10 | 0.74 | 0.45 | 0.15 | 0.60 | 2.74 | 10.3 | 1.66 | 29.4 |
| | 15 | 0.67 | 0.38 | 0.18 | 0.56 | 2.50 | 18.2 | 1.41 | 40.3 |
| | 20 | 0.66 | 0.37 | 0.18 | 0.55 | 2.43 | 20.5 | 1.35 | 42.6 |
| | 25 | 0.61 | 0.30 | 0.18 | 0.49 | 2.26 | 26.1 | 1.11 | 52.7 |
| | 30 | 0.59 | 0.26 | 0.21 | 0.47 | 2.18 | 28.6 | 0.97 | 59.0 |
| | slope | *−0.008* | *−0.012* | *0.003* | *−0.009* | *−0.030* | | *−0.045* | |
| | intercept | *0.81* | *0.60* | *0.12* | *0.72* | *3.02* | | *2.23* | |
| | RSQ | *0.983* | *0.960* | *0.882* | *0.930* | *0.983* | | *0.960* | |
| distillers dried grains and solubles, CP = 23.8% | 0 | 0.70 | 0.53 | 0.11 | 0.64 | 2.92 | 0.0 | 2.22 | 0.0 |
| | 5 | 0.63 | 0.41 | 0.11 | 0.52 | 2.63 | 10.1 | 1.73 | 22.1 |
| | 10 | 0.57 | 0.33 | 0.13 | 0.46 | 2.41 | 17.6 | 1.37 | 38.6 |
| | 15 | 0.55 | 0.28 | 0.14 | 0.43 | 2.30 | 21.3 | 1.19 | 46.3 |
| | 20 | 0.48 | 0.22 | 0.15 | 0.37 | 2.00 | 31.4 | 0.92 | 58.4 |
| | 25 | 0.47 | 0.21 | 0.14 | 0.35 | 1.98 | 32.2 | 0.89 | 60.1 |
| | 30 | 0.42 | 0.16 | 0.15 | 0.30 | 1.76 | 39.9 | 0.66 | 70.1 |
| | slope | *−0.009* | *−0.012* | *0.001* | *−0.010* | *−0.037* | | *−0.049* | |
| | intercept | *0.68* | *0.48* | *0.11* | *0.59* | *2.84* | | *2.01* | |
| | RSQ | *0.974* | *0.936* | *0.734* | *0.936* | *0.974* | | *0.936* | |

monia. Furthermore, its large peak prevents the determination of the residual lysine by overlapping in the chromatogram. This content, however, was important for us to check the balance of the guanidination reaction. Imbeah et al. (*14*) and Ravindran et al. (*15*) conducted investigations on the reaction yield as a function of the reagent excess. According to their results, 10:1 (casein), 12:1 (cottonseed protein), and up to 16:1 (soybean meal) are sufficient for complete reaction. They also achieved good yields with the lower concentration of 0.4 mol/L of OMIU reagent. To further confirm that our reaction conditions are optimal, we have sent three soy and three DDGS samples for comparison to the laboratory of coauthor Shane Rutherfurd. Our reactive lysine results were found to be between 102 and 106% of his laboratory's values for soy and between 121 and 129% for DDGS. The latter product was gunanidinated there using the same conditions as for soy, and they had never analyzed DDGS before. Thus, we believe that our reaction conditions are optimized well for both ingredients (see above).

**Lysine Damage through Autoclaving with Steam.** The results of the heat damage tests of soy products are represented in **Table 1**. The total lysine value determined according to conventional amino acid analysis decreases linearly with all three samples as a function of the heat treatment time with high RSQ values between 0.872 and 0.987. However, the reactive lysine content decreases much more (higher slope) with better RSQ values between 0.967 and 0.987. The residual lysine contents occurring after derivatization and hydrolysis increase also with high correlation to damage time to about 2.5 times the starting value. Most other essential amino acid contents (data not shown) indeed remained unchanged within a CV of about 2% with the exception of cystine and arginine, which are also partly destroyed. Thus, lysine damage is also observed with normal amino acid analysis with a decrease of 16.6−23.2%, although the determination of reactive lysine has higher sensitivity, showing a decrease of 34.7−41.4%.

**Figure 1** shows the analysis results of all soy samples divided by the respective crude protein content including the line for the sum of contents of reactive and residual lysine. In a perfect reaction balance Lys sum should be close to tLys. However, the Lys sum line is almost parallel to tLys with an interval of ca. 0.6% lysine/CP, which is missing in the reaction balance. All sample results fall together relative to CP contents. Leu/CP is also plotted and remains practically unchanged due to the high heat stability of this amino acid. The data of all three samples can also be represented differently by choosing the percentage of the loss of rLys/CP as the *x*-axis, as against the highest measured value of 5.19%. It is assumed here that the lysine content is genetically fixed and that deviations are mostly indicative of damage. In this representation (**Figure 2**) the rLys loss due to the heat treatment is quite obvious. Reactive Lys/CP has in this calculation a maximum loss of 43%.

The DDGS samples conducted with less-divergent heating times under analogous heat stress conditions show much greater damage than soy, presumably because of their sugar content (**Table 2**). Thus, total lysine decreases by 29 and 40%, respectively. In the latter case, the particularly heat-sensitive DDGS with CP = 24%, reactive lysine actually decreases by 70% due to 30 min of heating at 135 °C; in the other sample it decreases by 60%. The linear correlation of total and reactive lysine contents to the heating time is again very high with RSQ values between 0.936 and 0.983. Residual lysine is already quite high in the untreated samples, that is, 14–16%, as against total lysine content, but the increase is less than with soy. In the DDGS trial as well, the essential amino acid shows high heat stability (CV about 2%); only cystine and arginine are appreciably degraded. In most actual statistics of amino acid contents in feed ingredients (*12*) the average tLys/CP content in DDGS is 2.81%, with a wide range of variation of 1.38–3.60%. The DDGS samples of our tests have tLYS/CP values of 3.08 and 2.99%, values that decrease to 2.17 and 1.75%, respectively, after 30 min at 135 °C. Thus, we have used qualities somewhat damaged before, and all obtained results of the trial lie in the observed range of this new feed ingredient. In **Figure 3** the loss of rLys/CP in percent is chosen as the *x*-axis, calculated against the highest measured content here of 2.36%. The Lys sum/CP contents lie below the tLys/CP line, having a little higher slope with an increasing interval of ca. 0.2–0.5% Lys/CP missing in the reaction balance. Relatively seen, this gap is much larger than that with soy, with a reaction balance of ca. 90% against total lysine content for the briefly heated samples and of ca. 80% after 30 min in the autoclave. Included as a reference in the graph here is Val/CP, which is also practically unchanged due to the high thermal stability of

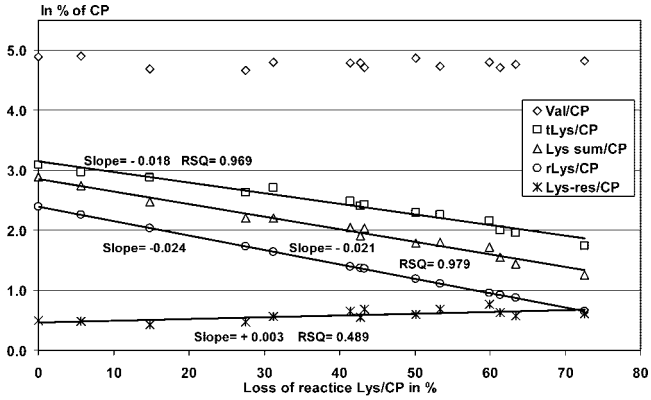


**Figure 3.** Heat treatment of the two DDGS qualities contents relative to crude protein dependent on the loss of rLys/CP. Up to 72% of reactive lysine is lost during the heat treatment.

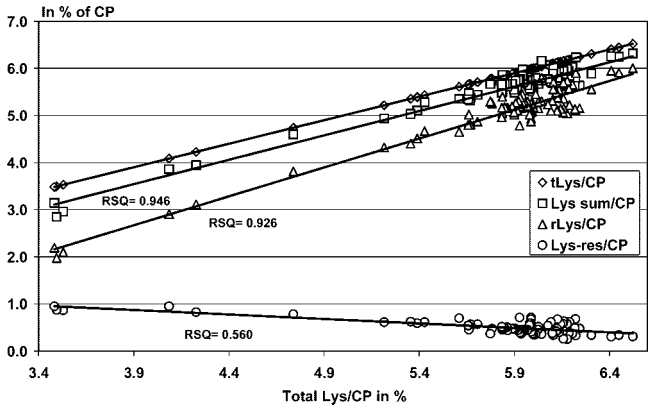


**Figure 4.** Determination of reactive lysine in 84 soy samples (full fat and extracted). Highest total and reactive lysine contents were found in untoasted beans and the lowest in black, overheated meals. There is also a gap between the total lysine and the sum of reactive and residual lysine from the guanidination reaction.

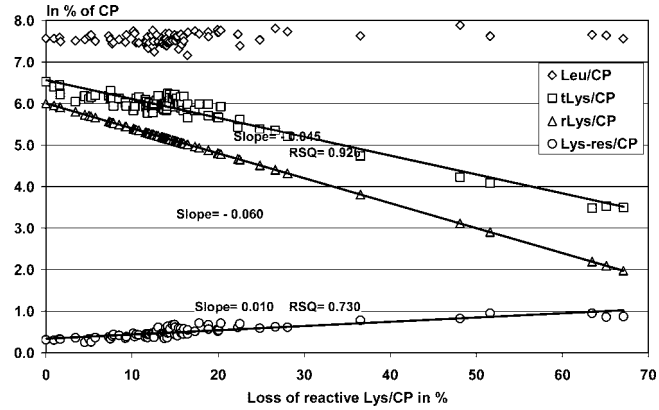


**Figure 5.** Results of the 84 soy samples displayed relative to the loss of rLys/CP, using the untoasted beans as reference. Common qualities show about 15% lower values than those.

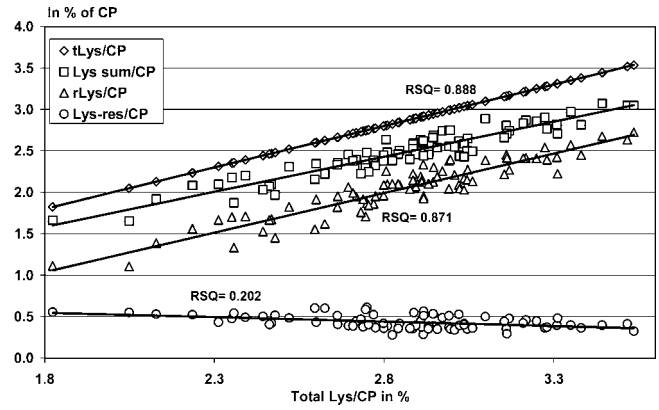


**Figure 6.** Determination of reactive lysine in 80 corn DDGS samples. There is a high variation in the contents. Declines of total lysine and reactive lysine are almost parallel. There is also an important gap between the total lysine and the sum of reactive and residual lysine from the guanidination reaction.

this amino acid. Reactive Lys/CP is clearly the most sensitive indicator of lysine damage, with a maximum of 72% loss in this evaluation of the autoclave studies.

**Results of Common Soy and DDGS Samples.** Eighty-four normal soy samples obtained from feed manufacturers were analyzed for reactive lysine. We had ordered some untoasted soybeans, which showed the highest lysine contents relative to crude protein as expected. However, they are not suitable for feeding, because soy protein must be toasted to improve the digestibility. Some of the samples were particularly dark-colored and sent on suspicion of heat damage during toasting. The amino acid composition of the protein is quite stable in soy; therefore, all results can be combined using contents relative to crude protein. **Figure 4** illustrates the results by using tLys/CP as the *x*-axis. Most samples have contents of $6 \pm 0.3\%$ tLys/CP, about 10% below the highest values for untoasted beans. Reactive Lys/CP and Lys-res/CP and their sum are linearly dependent on tLys/CP with RSQ values of 0.946, 0.560, and 0.926, respectively. This display corresponds well to the results of the autoclaving tests, declining reactive lysine is associated with increasing residual lysine, and the sum of both contents lies below the curve for tLys/CP with a constant interval. The content of rLys/CP is always below that of tLys/CP even for nontoasted soybeans.

The loss of rLys/CP relative to the highest observed value of 6.00%, found with untoasted beans, was calculated for each sample and used as the *x*-axis (**Figure 5**). The figure illustrates that 10–20% lysine damage is typical for normal soybean meals and that overheated batches had lost up to 67% of the reactive lysine. When the slopes and RSQ values are compared with the trial data of **Figure 2**, it is highly probable that heat damage is the reason for the obtained variability.

The 80 common corn-DDGS samples from U.S. production that we tested for reactive lysine have an average tLys/CP content of 2.86% with a CV of 11.5% and analyzed contents between 1.82 and 3.54%. Thus, they represent a good cross section of the typical variation of this raw material (see below). **Figure 6** illustrates the results displayed with tLys/CP as the *x*-axis. We find a wide distribution of the lysine contents. The rLys/CP contents are linearly dependent on tLys/CP (RSQ = 0.954). Decreasing rLys/CP is associated only with a flat increase in Lys-res/CP (RSQ = 0.202). The lines of tLys/CP and rLys/CP are nearly parallel, with a large interval, similar to the trial results. Also shown here is the sum of rLys/CP and Lys-res/CP. With a somewhat lower slope, this sum lies below the line for tLys/CP, on average at 84% of its figure with a standard deviation of 4%. For the batches with highest contents the rLys is 20% below the tLys content. Again, the loss of rLys/CP is calculated here on the basis of a maximum value of 2.73%. With loss of rLys/CP as *x*-axis **Figure 7** is obtained similarly to **Figure 3** of the autoclaving tests. The RSQ of tLys/CP to the loss of rLys/CP is 0.871, and its slopes have here very similar figures. The only slightly changing value of Val/CP proves to be a quite stable protein composition for all DDGS. This

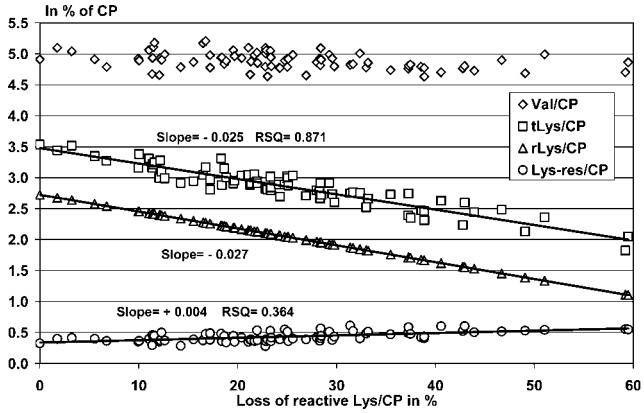


**Figure 7.** Results of the 80 DDGS samples displayed relative to the loss of rLys/CP, using the highest found result as reference. Almost half of the marketed qualities contain >20% less reactive lysine than best batches.

evaluation shows that 10–40% lysine damage is common for DDGS samples based on rLys results and that overheated batches can lose up to 59%. If the loss of tLys/CP is calculated, a common damage of 5–30% is found and up to 48% is found for the worst batches, thus being only somewhat less sensitive than with rLys values. Anyway, this ingredient is highly variable in lysine contents, and its use in large proportions represents a risk to the feed formulation.

**Discussion of Reaction Balance and Suitability of Reactive Lysine for Feed Evaluation.** Given the above results, characterization of the reactive lysine assay with regard to the possibility of secondary reactions and incomplete derivatization may be useful. The increase in residual lysine found after homoarginine reaction in the case of major damage is usually explained by the possible reverse degradation of damaged lysine (Maillard adducts) during acid hydrolysis (*1*), the reason for the flatter drop of the tLys/CP line. However, the presence of residual lysine in the untoasted soybeans may be due to incomplete guanidination, possibly as a result of the soy matrix. Alternatively, it is possible that the untoasted (but dried) soybeans contained damaged lysine derivatives as a consequence of drying. With more severe damage, a larger gap in the reaction balance of soy products is found with increasing residual lysine and decreasing reactive lysine, averaging a 5% gap for all samples and 10–19% for the most severely damaged samples. In heat stress tests (**Table 1**) there was also a certain decline of the balance with more heat damage. The gap found for lysine could be explained by the assumption that part of the damaged lysine is released again during direct acid hydrolysis, but under the alkaline guanidination conditions, it can further react to no longer reverse-degradable derivatives and, therefore, less residual lysine is formed during subsequent acid hydrolysis.

Although DDGS is based on a vegetable raw material, it is more variable than soy in its amino acid composition because of its yeast protein content from alcoholic fermentation. During alcohol distillation and drying, major thermal stress can occur, and this can readily cause Maillard reactions (*1*) of the protein-bound lysine due to the presence of sugar. Thus, according to ref *12*, the CV of the crude protein in 409 analyzed DDGS samples is only 5.0%, with 22.4−30.8% CP content, whereas tLys/CP averages 2.80%, with a large CV of 13.0%, from 1.38 to 3.60% analyzed content. The DDGS raw material corn itself has an average tLys/CP of 3.12% with a CV of only 6.5% and contents between 2.45 and 3.80%.

In the case of DDGS, untreated reference samples are not available as they are with soy. However, we can see in the autoclave tests that the reaction balance also becomes poorer with increasing lysine damage. Thus, it is 93% in the case of starting products, but decreases to 73–81% after strong thermal stress. As above, this could be explained by the fact that existing acid-reverse-degradable lysine derivatives further react to irreversible adducts due to the alkaline conditions of the homoarginine reaction.

In the analysis of the 80 DDGS samples, however, this is not so evident (**Figure 6**). Here as well, the interval between tLys/CP and rLys/CP is almost parallel and very large. As there is a high correlation between rLys and tLys also, the total lysine content by normal amino acid analysis indicates damage well. The Lys-res content, which increases little on the whole in contrast to soy, could partly be caused by incomplete reaction of the undamaged lysine to homoarginine in the protein; however, this should imply a close to 100% reaction balance. In fact, it ranges from 93% to only 80% with no evident correlation with the rLys value as in the case of soy. This gap of at least 7% indicates that even the best DDGS qualities at the market have an important portion of heat-damaged lysine. In view of the results, the advantage of the reactive lysine over the total lysine for determining protein quality in DDGS may be limited. A classification of DDGS batches by analyzing tLys/CP (above 3.2%, good; below 2.3%, badly damaged) seems to provide sufficient information for the animal nutritionist.

Rutherfurd and Moughan (*16*) have also applied heat stress to skim milk powder in 1–10 min autoclaving times at 121 °C and to peas by heating them for 15 min at 110, 135, 150, and 165 °C in an oven. For skim milk powder total and reactive lysine contents were identical for the unheated product, but tLys decreased by 33% of the original value after 10 min of heating, whereas the rLys decreased by 82%. Reactive lysine showed the damage much more sensitively, because a big part of the Maillard adducts is cleaved back to lysine during acid–protein hydrolysis. Unpublished results of these residual lysine contents showed indeed that the gap between Lys sum and tLys greatly increases with heat damage. Starting at zero difference (100% reaction balance) the gap increased to 17, 29, 41, and 56% relative to tLys at the different heating times. We conclude that during increased heating Maillard products are formed, which further react under alkaline conditions to nonreversible adducts, whereas they can cleave back to lysine directly heated with hydrochloric acid in hydrolysis. For the peas, however, the situation was different. The rLys content of the unheated sample was 98.7% of the tLys, and both go strongly decrease after oven heating. The tLys decreased by 42% in the sample treated at 165 °C and rLys by 64%; for the sample heated at 150 °C the effect is only –20 to –29%. Unpublished results show here that the sum of residual and reactive lysine always equals total lysine; thus, a perfect reaction balance is observed. It seems that in heat-stressed peas lysine derivates are formed, which are not influenced by the alkaline guanidination conditions. However, Lys-res strongly increases from only 4% relative to tLys in raw peas to 41% in the sample treated at 165 °C.

It is therefore obvious that each raw material responds differently to the guanidination reaction and that the solubility of its protein and its sugar content and type play important roles. A perfect reaction balance cannot be expected especially after strong heat stress as there are many different pathways by which lysine can react to unavailable derivates. Given that soy and DDGS are processed protein sources, we can never be sure that the protein-bound lysine is completely converted to homoargi-

nine during guanidination, and the possibility that undamaged lysine is not completely guanidinated in some feedstuffs should not be overlooked. Possibly the reactive lysine could be somewhat smaller than the available, undamaged lysine. However, reactive lysine is closer to available lysine than the total content after usual hydrolysis and is a more sensitive indicator of heat damage in feed ingredients.

## ABBREVIATIONS USED

OMIU, *o*-methylisourea, hydrogen sulfate; DDGS, distillers dried grains with solubles (corn basis); CP, crude protein; Lys, lysine; tLys, total lysine content; rLys, reactive lysine content; Lys sum, sum of the reactive and residual underivatized lysine content after guanidination; Lys-res, residual lysine after guanidination reaction; Leu, leucine; Val, valine; CV, coefficient of variation = relative standard deviation; CVr, coefficient of variation = relative standard deviation as within laboratory repeatability; RSQ, coefficient of determination = square of linear regression correlation *r*.

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