Variability and Relationships among Amino Acids and Nitrogen in Maize Grains

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The amino acid (AA) composition of 30 samples of maize (*Zea mays*) grains from 13 different varieties was determined with maximum accuracy (four different acid hydrolyses plus tryptophan and amide nitrogen determinations). Total nitrogen contents (*N*) ranged from 1 to 3% of grain dry matter. On a dry matter basis, the levels of each AA increased linearly as a function of *N* with correlation coefficients often higher than 0.98. For a given AA, the same linear relationship was obeyed for the samples resulting from different phenotypes as well as from different genotypes. Hence, the AA composition of any maize grain sample can be predicted from its *N*. On a protein basis, AA contents changed as hyperbolic functions of *N*. The same was true for the degree of amidation increasing from 66 to 82%, the nonprotein nitrogen to total nitrogen ratio decreasing from 9.9 to 3.2% while the nitrogen to protein conversion factor remained constant (5.77) within the range investigated.

In spite of considerable use of maize for food and feed in different parts of the world, complete and accurate amino acid (AA) composition of its grain is not available except in single samples like those analyzed by Wu and Sexson (1976) and by Pedersen and Eggum (1983). The same is true for variation of AA composition of maize grain according to the amount of total proteins deposited i.e. according to nitrogen content (*N*) in percent of grain dry matter. Several studies still have investigated the question by analyzing grain samples differing more or less in their *N* and resulting either from different environmental conditions (in particular nitrogen ferilization) or from different genotypes, or from both kinds of influence. MacGregor et al. (1961) showed that fertilization increased AA level of grain, but they noticed that the concentration in total protein of some AAs substantially decreased when *N* increased from 1.2 to 1.8. In the same range of *N*, Bressani et al. (1962) found that correlations between *N* and AA levels were significant only with some AAs, and for instance not with lysine. On the contrary, Paez et al. (1969) found a significant correlation between *N* (varying from 1.6 to 2.6) and lysine in grain of 48 selected strains but only little variability for inbred lines grown at different stations and years. In the same *N* range, Davis et al. (1970) analyzed 114 samples of inbred grains. They found significant relationships among AA concentrations (as well percent of sample as percent of protein) but concluded that further study may explain more dependent variables by including additional independent variables in the regression analyses. Keeney (1970) made an investigation similar to the work of MacGregor et al. (1961) in a little lower *N* range (from 1.04 to 1.57). He confirmed that the level of almost all essential AAs was highest in the grain having the highest *N*. Gevers (1975) suggested a possible correlation between lysine and tryptophan and insisted on the importance to use accurate analytical methods. Rendig and Broadbent (1979) compared grain samples of two different *N* (0.88, 1.54) resulting from various levels of applied nitrogen. They confirmed positive or negative differences in the AA concentrations in protein according to AA. The same conclusions were also drawn by Imamul Huq (1983) on eight samples of a local variety differing by their *N* (1.4–2) and resulting from different fertilizer applications. Like Davis et al. (1970), Imamul Huq (1983) found significant correlation between *N* and AA concentration (in sample as well as in protein) but he did not draw any conclusion. Therefore, the accurate nature of the relationships involved was not clarified: no clear data are available, as well on the magnitude of the variation of each AA concentration in grain according to *N*, as on the exact nature of this variation of AA composition as a function of *N*. The narrowness of *N* range investigated, the small number of grain samples involved in many studies, and especially the great dispersion of analytical results are generally the chief reasons resulting in such a confused situation. This paper tentatively provides some of these missing data. In this aim, the grain samples investigated ranged from very low- to high-*N* samples for several different varieties. Moreover, all protein AAs have been analyzed with the highest accuracy obtainable today. Tkachuk (1966) underlined how essential it is to carry out analyses after several different hydrolysis times. However, this entails so much extra work that as far as we know all studies published until now result from analyses based on single hydrolysis times (generally 24 h). Thus, in the present work all the AA data are based on determinations using four different hydrolysis times, except for tryptophan and amide nitrogen, which are analyzed after two extra different hydrolyses.

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EXPERIMENTAL SECTION

Material. The grain samples investigated arose from field-grown corns. They were selected in order to cover the widest range of protein content available. They included one inbred [*IHP*, Illinois high protein] and 12 commercial hybrids [*Axia*, *80B97*, *Pride 1108*, *Dea*, *Liza*, and *FCS 10400*, each represented by several (from two to five) samples covering more or less wide range of grain protein content, and *Concorde*, *LG1*, *Bruex*, *Roc*, *Ogalo*, and *Fulvia*, of which only single samples were studied]. The 12 hybrids correspond to different kinds of corns recently cultivated in Europe. Grain sampling (from 1 kg), grain milling, and meal subsampling for analysis were performed in the conditions already described elsewhere (Mossé et al., 1985).

Analytical Methods. Moisture was determined in triplicate and also total nitrogen by micro-Kjeldahl. Amide nitrogen (giving the amount of asparagine plus glutamine) corresponded to titrated free NH_3 resulting from a short hydrolysis (3 h in 2 N HCl at 115 °C). AAs were determined from analyses made by single column chromatography with four different 6 N HCl hydrolysates as to account for losses due to either degradation or incomplete release: 15, 24, and 48 h plus an 18-h hydrolysis of a

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Table I. Amino Acid Composition of Maize Samples (mg/100 g of Grain Dry Matter, i.e. $A_i \times 10^3$)

var[a]	Axi	Axi	Pri	Roc	Pri	Ful	Pri	Dea	Dea	Liz	Axi	FCS	Axi	FCS	Axi
N[b]	1.04	1.06	1.13	1.14	1.17	1.22	1.23	1.34	1.36	1.37	1.37	1.46	1.46	1.48	1.63
Gly	294	299	296	317	285	322	304	333	342	354	330	378	330	384	371
Ala	466	457	511	490	529	542	551	601	626	621	611	696	696	666	785
Val	334	343	360	359	365	366	377	445	457	437	420	469	458	467	480
Leu	694	682	790	779	818	825	886	1008	1031	1034	1002	1092	1159	1068	1357
Ile	235	235	268	262	266	254	285	323	316	321	307	341	343	338	377
Ser	311	307	353	352	354	350	385	411	424	429	410	478	456	467	536
Thr	248	246	282	267	281	283	300	322	328	315	306	355	333	356	383
Tyr	272	272	286	288	292	330	308	366	383	353	358	373	392	370	428
Phe	293	292	317	325	321	348	347	397	411	411	410	432	464	426	510
Trp	51	54	47	57	46	54	46	62	62	60	60	63	63	64	60
Pro	599	617	624	672	672	689	685	769	853	860	787	810	875	814	896
Met	137	181	176	159	168	167	204	171	172	161	178	196	170	204	189
$^1/_2$ Cys	186	189	194	206	175	234	193	216	231	237	224	237	214	236	219
Lys	235	244	212	257	186	263	204	267	273	288	268	314	275	315	275
His	189	186	208	222	223	215	223	252	269	262	242	282	252	282	270
Arg	350	348	342	389	318	395	347	397	414	454	416	478	431	481	479
Asx[c]	436	454	455	455	442	491	485	514	549	557	530	619	581	620	683
Glx[c]	1073	1111	1220	1226	1278	1278	1328	1535	1587	1555	1532	1702	1734	1684	1994
amide NH_3[d]	117	122	141	132	150	152	162	181	183	175	180	200	206	204	245
N recd, %	91.55	91.5	91.4	91.7	89.1	91.05	89.7	91.95	94.9	94.75	91.2	95.7	93.2	93.8	93.8

[a] Axi = Axia; Bru = Bruex; Con = Concorde; Dea = Dea; FCS = FCS 10400; Ful = Fulvia; IHP = Illinois high protein; LG1 = LG1; Liz = Liza; Oga = Ogalo; Pri = Pride 108; Roc = Roc; 80B = 80B97. [b] N = grain nitrogen content (grams/100 g of dry matter). [c] Asx and Glx

sample previously oxidized by performic acid. An extra hydrolysis in $Ba(OH)_2$ allowed the determination of tryptophan in triplicate. All the methods used for AA analysis have been already detailed elsewhere (Mossé et al., 1985). Lipid content was determined by hexane (8 h) in a Soxhlet extrator.

RESULTS

The average weights of grain ranged from 220 to 350 mg. Lipid content ranged from 3 to 5 g/100 g of grain dry matter except for the two samples of variety *FCS 10400*, which was selected for its high lipid content (8 and 8.1 g/100 g of grain dry matter). On a moisture-free basis, N ranged from 1.04 to 2.95 for the whole set of 30 samples (Table I). The range of N covered by the different samples of a given variety was near 0.5 ± 0.1 (in grams/100 g of grain dry matter) except for *IHP* samples. For instance, for *Axia*, *Dea*, *Liza*, and *80B97* samples, the N ranged from 1.04 to 1.45, 1.34 to 1.75, 1.37 to 1.86, and 1.63 to 2.08 g/100 g of grain dry matter, respectively. It can be noted that these ranges overlapped one another and that the six varieties represented by single samples were also distributed inside these ranges. However, the four *IHP* samples had the highest N (2.31–2.95), which did not overlap those of any other 26 samples studied.

Variability of AA Contents of Grain according to N. For AA and nitrogen, analytical data obtained for the 30 samples are given in Table I. In this table, AA composition is expressed in grams of AA/100 g of grain dry matter (i.e., A_i). When plotted against N, variations of A_i were represented by straight lines for each AA and also for amide ammonium arising from amide groups of glutamine and asparagine (not shown). Hence, the variations of A_i can be described by the equation 1

$$A_i = a_i N + b_i \qquad (1)$$

where a_i is the slope of the line corresponding to the ith AA and b_i its intercept with the ordinate. It can be noted that a_i gives the increment of A_i for a given increase of N. It is always positive, whereas b_i can be positive, equal to zero, or negative according to the AA. Thus, it was possible to calculate from the AA compositions in Table I the values of coefficients a_i and b_i from the regression lines for each AA and the corresponding correlation coefficients (Table II). The correlation coefficients were greater than 0.97 for 14 AAs and for amide nitrogen. The values ranged from 0.94 to 0.96 for tryptophan, methionine, and half-cystine. Lysine had the lowest correlation coefficient (0.894). These last four AAs are well-known for the analytical difficulties encountered in their determination.

Table II. Slope (a_i ± SE), Intercept (b_i ± SE), and Correlation Coefficient (r_i) of Regression Lines Representing Amino Acid Content (A_i) as a Function of Nitrogen Content (N), When A_i and N Are Expressed in Grams/100 g of Grain Dry Matter

amino acid	a_i ± SE	b_i ± SE	r_i[a]
Gly	0.145 ± 0.007	0.137 ± 0.012	0.970
Ala	0.576 ± 0.009	−0.160 ± 0.015	0.997
Val	0.291 ± 0.007	0.034 ± 0.012	0.992
Leu	1.110 ± 0.017	−0.491 ± 0.030	0.997
Ile	0.245 ± 0.007	−0.019 ± 0.011	0.990
Ser	0.362 ± 0.005	−0.070 ± 0.009	0.997
Thr	0.217 ± 0.004	0.024 ± 0.007	0.995
Tyr	0.282 ± 0.007	−0.028 ± 0.012	0.991
Phe	0.381 ± 0.006	−0.113 ± 0.010	0.997
Trp	0.025 ± 0.002	0.0237 ± 0.003	0.949
Pro	0.571 ± 0.017	0.008 ± 0.029	0.988
Met	0.099 ± 0.007	0.047 ± 0.011	0.943
$^1/_2$ Cys	0.119 ± 0.007	0.053 ± 0.012	0.958
Lys	0.099 ± 0.009	0.130 ± 0.016	0.894
His	0.143 ± 0.006	0.056 ± 0.010	0.979
Arg	0.199 ± 0.010	0.143 ± 0.017	0.968
Asx	0.415 ± 0.012	−0.012 ± 0.021	0.989
Glx	1.568 ± 0.014	−0.584 ± 0.023	0.999
NH_3	0.204 ± 0.004	−0.095 ± 0.007	0.995

[a] Significance level of r_i: 0.570 (p = 0.001).

Variability of AA in Crude Protein according to N. As the AA content C_i of crude protein (in grams/16 g of seed nitrogen) is related to A_i by $C_i = 16\,A_i/N$, it follows that

$$C_i = 16a_i + 16b_i/N \qquad (2)$$

Hence, C_i is related to N by a second-degree equation represented by a segment of equilateral hyperbola (Figure 1). Its vertical asymptote coincides with the ordinate axis. Its horizontal asymptote has an ordinate equal to $16a_i$. Therefore, the AAs of maize grain can be distributed in

80B	LG1	Liz	Con	Dea	Oga	Liz	Bru	80B	80B	80B	IHP	IHP	IHP	IHP
1.63	1.67	1.73	1.74	1.75	1.77	1.86	1.94	1.98	2.04	2.08	2.31	2.46	2.64	2.95
367	355	373	391	395	386	401	431	408	402	416	477	531	545	554
801	794	824	825	832	886	899	960	969	975	1027	1214	1247	1320	1596
515	528	550	499	591	572	581	601	599	612	624	730	732	792	895
1321	1364	1434	1411	1404	1517	1602	1652	1704	1766	1846	2139	2202	2302	2878
383	392	414	396	422	445	452	443	457	491	488	499	562	611	747
526	528	546	559	534	558	599	656	661	663	689	751	801	875	1024
383	376	399	391	402	396	437	468	451	453	468	549	562	586	664
428	434	494	434	480	472	532	480	519	548	572	650	662	685	821
520	517	558	543	550	578	617	644	636	657	689	751	816	858	1042
65	63	70	66	63	64	67	73	67	67	78	82	86	92	99
882	888	1061	982	1109	1052	1125	1094	1118	1148	1170	1337	1432	1460	1725
219	188	215	201	204	197	223	267	259	274	254	282	300	297	341
235	235	257	244	263	231	278	267	290	293	273	332	347	380	443
322	319	297	309	299	314	309	322	315	319	306	368	400	413	387
286	303	315	304	329	290	338	334	359	357	364	390	408	429	443
469	475	471	494	504	496	490	516	531	529	546	636	662	677	683
719	747	664	705	666	768	733	772	840	803	832	946	1016	1031	1255
1999	2017	2083	2159	2074	2218	2322	2442	2526	2601	2672	3013	3311	3531	4105
255	256	254	255	246	265	285	279	327	312	351	368	416	454	498
95.2	94.2	94.15	93.2	93.9	95.25	94.8	93.9	95.7	93.5	95.4	96.6	96.7	95.4	97.1

were evaluated in terms of grams of Asp and Glu. [d]Amide nitrogen was evaluated in term of grams of ammonia. It gives an estimation corresponding to the sum (Asn + Gln), i.e. asparagine plus glutamine.

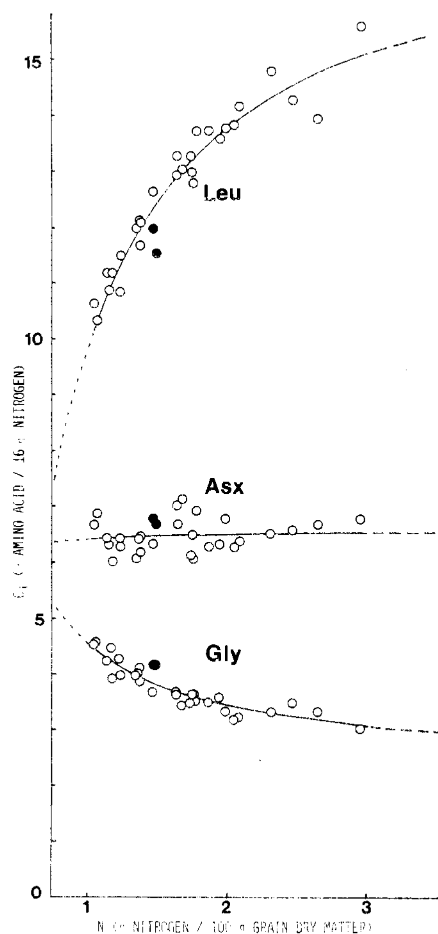

Figure 1. Relationship between amino acid content, C_i, of crude protein (grams/16 g of nitrogen) and nitrogen content N for leucine (Leu), asparagine + aspartic acid (Asx), and glycine (Gly). Key: black circles, high-lipid content variety FCS 10400; open circles, all other varieties.

three different groups according to the sign of b_i and the magnitude of the ratio b_i/a_i.

A first group corresponds to AAs for which b_i/a_i is negative and nonnegligible. In such conditions, C_i increases as a function of N. It increases much more for low- than for high-nitrogen grains. Theoretically, it tends toward a limit value that is the ordinate of the horizontal asymptote of the hyperbola. This is the case for alanine, leucine (Figure 1), serine, phenylalanine, and glutamine plus glutamic acid.

A second group corresponds to AAs for which b_i/a_i is negligible. According to eq 2, the segment of hyperbola representing C_i as a function of N is very close to a straight horizontal line. Such is the case (Figure 1) for Asx (asparagine plus aspartic acid) for which $b_i/a_i = -0.029$ g of nitrogen/100 g of grain. That implies that maize grain proteins have the same level of Asx, $C_{Asx} \sim 16a_{Asx} = 6.6$ g/16 g of nitrogen whatever N may be. As b_{Asx} is also negligible (Table II), eq 1 shows that A_{Asx} is proportional to N. Such is also the case for five other AAs: valine, isoleucine, threonine, tyrosine, proline.

A last group corresponds to AAs for which b_i/a_i is positive and nonnegligible. For instance, as N increases, the value C_{Gly} for glycine in crude proteins drops significantly in the lower range of N, but it decreases more slowly in the higher range (Figure 1). The same is true for six other AAs: tryptophan, methionine, half-cystine, lysine, histidine, arginine.

From a nutritional or practical viewpoint, it is interesting to quantify the variations of C_i in the range investigated according to the AA. In Table III are reported the calculated values of C_i for N = 1–3, respectively, and also the ratio of the difference $C_i(N = 3) - C_i(N = 1)$ to $C_i(N = 2)$, giving an estimation of the relative variation of C_i in the range investigated. Glutamine plus glutamic acid and leucine are those that exhibit the highest (positive) variations of C_i as a function of N. Alanine and phenylalanine also show significant increases and glycine, lysine, and arginine significant decreases. But, the relative variations are the highest with lysine, glycine, and tryptophan.

Variability in Degree of Amidation of Maize Proteins. The AA level $B_i = A_i/M_i$ expressed in mole number of AA/100 g of grain dry matter (M_i being the molecular weight of the ith AA) is also a linear function of N and can be calculated from Table II. As B_{NH_3} gives the total number of moles of amidated AA, the degree of amidation of maize grain protein, e.g. the molar ratio (Asn + Gln)/

Table III. Predicted Amino Acid Composition C_i of Maize Grain[a] (in Gram/16 g of Nitrogen) for Three Different N and Maximal Relative Difference $\Delta C_i/C_i$[b]

amino acid	nitrogen content N, % dry matter			$\Delta C_i/C_i$,[b] %
	1	2	3	
Gly	4.5	3.4	3.05	-42.6
Ala	6.65	7.95	8.35	21.4
Val	5.2	4.95	4.85	-7.1
Leu	9.9	13.8	15.1	37.7
Ile	3.6	3.75	3.8	5.3
Ser	4.65	5.25	5.4	14.3
Thr	3.85	3.65	3.6	-6.8
Tyr	4.05	4.3	4.35	7.0
Phe	4.3	5.2	5.5	23.1
Trp	0.78	0.59	0.53	-42.4
Pro	9.25	9.2	9.2	-0.5
Met	2.35	1.95	1.85	-25.6
$^1/_2$ Cys	2.75	2.35	2.2	-23.4
Lys	3.65	2.6	2.3	-51.9
His	3.2	2.75	2.6	-21.8
Arg	5.45	4.35	3.95	-34.5
Asx	6.45	6.55	6.6	2.3
Glx	15.7	20.4	22.0	30.9
NH_3	1.75	2.5	2.75	40.

[a] Calculated values from eq 2 with the coefficients given in Table II were approximated according to AA analysis accuracy. [b] $\Delta C_i/C_i = 100[C_i(N = 3) - C_i(N = 1)]/C_i(N = 2)$ in the range investigated.

(Asx + Glx) can be calculated as a function of N. This molar ratio is

$$B_{NH_3}/(B_{Asx} + B_{Glx}) = (1199N - 558)/(1377N - 406) \quad (3)$$

This relation shows that the degree of amidation of protein increases with N. For instance, it increases from 66 to 81.6% when N increases from 1 to 3. This shows that the degree of amidation of maize grain protein changes significantly according to N, contrary to what occurs with wheat (Mossé et al., 1985).

Variability of the Nitrogen to Protein Conversion Factor. The weight of AA residues (in grams/100 g of grain dry matter) is given by $E_i = (M_i - 18)A_i/M_i$ of which the sum $\sum E_i$ (amide ammonia being not included) is equal to the weight of true proteins/100 g of grain dry matter. Approximating E_{Asx} and E_{Glx} to E_{Asn} and E_{Gln}, respectively, and regressing $\sum(M_i - 18)A_i/M_i$ vs. N for all samples lead to $\sum E_i = 5.88N - 0.71$. Therefore, the conversion factor $k_p = (\sum E_i)/N$ for total Kjeldahl nitrogen to true protein is

$$k_p = 5.88 - 0.71/N \quad (4)$$

This relationship shows that k_p increases as a hyperbolic function of N from 5.17 to 5.64 when N rises from 1 to 3.

Another possible definition of this coefficient is the most frequently used ratio k_A of true proteins to nitrogen recovered from AA analyses (Tkachuk, 1966, 1969). Let $D_i = 14n_iA_i/M_i$ be the AA level expressed in grams of AA nitrogen/100 g of grain dry matter (n_i being the number of nitrogen atoms per molecule of the ith AA). Nitrogen recovered through AA analyses is given by the sum $\sum D_i$ (including amide nitrogen). It is easy to see that $k_A = (\sum E_i)/(\sum D_i)$ where $\sum E_i$ does not include the amide ammonia, whereas $\sum D_i$ does. Calculation shows that

$$k_A = (5.88N - 0.72)/(1.001N - 0.100) \quad (5)$$

It increases slowly as a hyperbolic function of N, from 5.73 to 5.81 when N rises from 1 to 3. That means that it can be considered as roughly constant and equal to 5.77 within 0.04 unit.

DISCUSSION

The most complete AA composition (including tryptophan determination) of maize grain has been published by Pedersen and Eggum (1983). These authors analyzed a maize sample corresponding to 9.9% of crude protein ($N \times 6.25$), i.e. $N = 1.584$. For the same N, AA composition calculated by the use of eq 1 with data reported in Table II is given in Table IV beside the results of Pedersen and Eggum (1983). Table IV shows that both compositions are very close to each other, except for some of the few AAs remaining always difficult to determine with accuracy. For instance, 12 AAs corresponded to relative deviations lower than 5% and the highest deviations reached nearly 15% for three AAs: tyrosine, half-cystine, tryptophan. It is striking to note that these deviations are equal to or lower than those described in an interlaboratory comparison of AA analyses by Sarwar et al. (1983).

In a study of many inbred grain samples with N ranging from 1.6 to 2.6, Davis et al. (1970) gave regression equations with coefficients homologous to those reported in Table II. When expressed in the same way, many values of Table II do not agree with those calculated by Davis et al. (1970). But, the coefficients of correlation r_i reported in Table II are considerably higher than the values (from 0.25 to 0.51) found by these authors. These discrepancies can be explained by taking into account the improvement of the technique of AA analysis during the last 15 years and also by the number of determinations made on each sample after different hydrolyses in the present work.

From an analytical standpoint, the determination of amide nitrogen allows the calculation of the yield $Y = (\sum D_i)/N$ of AA analyses, e.g. the ratio of total nitrogen ($\sum D_i$) recovered through AA analyses (see Results) to total grain nitrogen determined by the Kjeldahl procedure. Calculation shows that $Y = 1.001\ (\pm 0.007) - [0.100 \pm (0.013)]/N$. This means that Y increases as a function of N according to a hyperbolic law. Roughly speaking, its values are 0.90, 0.95, and 0.975 for N = 1, 2, and 3, respectively. This results from the presence of nonprotein nitrogen (NPN) compounds, mainly nucleic acids, not determined by the technique used in AA analysis and possibly free nonprotein AAs. Christianson et al. (1965) found that free nonprotein AA does not exceed 0.1% of total grain nitrogen. For other kinds of NPN compounds not determined by AA analysis, calculation shows that their level $D_{NPN} = N(1 - Y)$ is nearly constant, whatever N may be in the mature grain and close to 0.1 g of nitrogen/100 g of grain dry matter. This also shows that the substitution of total nitrogen for protein nitrogen appears quite acceptable for the relations between AA and N in maize.

As long as NPN amount can be neglected compared to protein nitrogen amount, the occurrence of relationships between one AA content and another or between each AA and N is an obviousness even though it has been sometimes questioned or controverted (Bressani et al., 1962; Davis et al., 1970; Dumanovic and Denic, 1969; Gevers, 1975): when proteins are deposited in grain, the level of protein AA rises, which results into an increase of N. As these AAs are bound in polypeptide chains, most of which contain each of the 20 protein AAs, it stands to reason that the variations in the content of one of them are correlated with the variations of each of the other ones.

The present results show that it is A_i, and not C_i, which changes linearly as a function of N, whatever the variety of maize. This suggests that any condition that can give rise to a modification of N, whether its origin be genetic or not, leads to the same modifications in AA composition.

Table IV. Amino Acid Composition of a Maize Grain (A_i, g of Amino Acid/100 g of Grain Dry Matter): Comparison between Experimental and Predicted Values

amino acid	exp[a]	pred[b]
Gly	0.38	0.37
Ala	0.74	0.75
Val	0.48	0.49
Leu	1.23	1.27
Ile	0.35	0.37
Ser	0.50	0.50
Thr	0.35	0.37
Tyr	0.37	0.42
Phe	0.45	0.49
Trp	0.07	0.06
Pro	0.86	0.91
Met	0.20	0.20
$^1/_2$ Cys	0.21	0.24
Lys	0.29	0.29
His	0.28	0.28
Arg	0.47	0.46
Asx	0.64	0.65
Glx	1.87	1.90

[a] Experimental data from Pedersen and Eggum (1983) who investigated a yellow maize U.S. grade No. 3. [b] Predicted values from eq 1 and Table II for the same nitrogen content: N = 1.584%.

This means that AA composition exclusively depends upon N, just like it has been found in wheat grain (Mossé et al., 1985). Conversely, once the data in Table II are available, the knowledge of N in a maize grain sample allows the calculation of its content in any protein AA. Moreover, the same is true for any grain sample that could be a mixture of grains either of different varieties or of different N. The average AA composition may be calculated from the average N determined on a mixture of grains (after suitable homogenization).

The question as to whether or not exceptions to the linearity between A_i and N can occur is worth discussing. It is evident that relationships found for normal maize are not valid for biochemical mutants. In barley, AAs of mutants like Hiproly have been shown to vary according to the same kind of linear relationships than normal barley with still different coefficients a_i and b_i (Mossé and Baudet, 1977). This suggests that biochemical mutants behave just like new species.

Another reason to expect a nonlinearity between A_i and N could arise from an exceptional large size of germs. It is well-known that the germ of normal maize is about 12% of kernel weight (Inglett, 1970) with possible variations from 9 to 19% (Bjarnason and Pollmer, 1972). Whatever its size may be, its composition appears as very stable. It is much richer in lysine than endosperm: around 5.6 g/16 g of nitrogen according to Garcia et al. (1972). It is also the main source of kernel oil, which amounts to about 5% (Reiners and Gooding, 1970; Jugenheimer, 1976). Therefore, two samples of a large germ variety, *FCS 10400*, known for its high oil content (8%) were inserted among the 30 grain samples analyzed in the present work. It can be seen in Figure 1 that the black points corresponding to this high-oil variety are practically the most distant from the regression curves of leucine and glycine. But the difference between A_{Lys} found for these two samples and predicted A_{Lys} is not statistically significant. Therefore, the predicted values in Table II remain valid with a good approximation even for large-germ grains of commercial hybrids.

In very particular conditions, the sulfur starvation appears as the only known factor able to result in a breakdown in the linear relationships described here. In plants supplied with low levels of sulfur (just sufficient to allow grain development), serious disturbances occur. For instance, barley grains suitably deprived of sulfur were shown not to obey the linear relationships (Mossé and Baudet, 1969). In such conditions of sulfur starvation, it was shown with barley (Eppendorfer, 1975), wheat (Byers and Bolton, 1979), and maize (Baudet et al., 1981) that the relative proportions of the main protein groups were changed, resulting in a modification of the whole AA composition. But if these very exceptional conditions are excepted, the present results suggest that all the environmental factors known to be capable of changing N appear to induce modifications in the AA level according to the relations reported in Table II, which enable to predict the AA composition of any commercial maize grain from its N only. This could spare in the future many AA analyses. This could be helpful for a better fitting of nutritional quality in food and feed industries. It might also increase the interest of giving a consideration to any payment of grain quality to farmers according to the N (or protein content) of crops.

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Functional Properties of Cross-Linked Lysozyme and Serum Albumin

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The importance of the flexibility of protein molecules in determining their foaming and emulsifying properties was investigated using egg white lysozyme and bovine serum albumin. Samples of the two proteins, which differ in their flexibility and functional properties, were cross-linked intramolecularly by treatment with iodine or a diphenyl sulfone reagent. The properties of native and treated proteins were compared. The monomeric cross-linked proteins were resistant to heat-induced conformational changes, as indicated by circular dichroism, and were also resistant to hydrolysis by chymotrypsin. These results indicate reduced flexibility. The cross-linking greatly reduced the foaming power and foam stability of both proteins. There was a similar but less marked change in the emulsifying activity and emulsion stability. The findings confirm the importance of protein flexibility in determining foaming and emulsifying properties of proteins.

INTRODUCTION

Since the determination method of protein surface hydrophobicity was established (Kato and Nakai, 1980), a number of studies (Kato et al., 1983a; Nakai, 1983; Shimizu et al., 1983) have been done on the relationship between protein structural and functional properties such as emulsifying and foaming properties. It is reasonable to assume that protein surface hydrophobicity plays a governing role in emulsification and foaming, because amphiphilic proteins possessing high surface hydrophobicity are strongly adsorbed at the interface between oil or air and water to cause a pronounced reduction of interfacial or surface tension that readily facilitates emulsification and foaming. There are apparent relationship between the hydrophobicity and functional properties, but other factors are also involved in addition to this characteristic. We have reported that the flexibility of protein structure detected by protease digestion method is also an important structural factor governing the emulsion and foaming (Kato et al., 1985). If proteins, though the surface hydrophobicity is originally low, are susceptible to denaturation at the oil–water and air–water interface, the surface hydrophobicity may increase at the interface to result in good foaming and emulsifying properties.

The flexibility of proteins can be defined as the denaturation equilibrium under physiological conditions. A considerable $N \rightleftharpoons D$ transition in proteins is observed even under physiological conditions. The equilibrium constant (K_D) for the $N \rightleftharpoons D$ transition in native proteins is in the range of about 10^{-5}–10^{-6}. Even if the K_D value increases in the range of 10^{-2}–10^{-3} under mild denaturation conditions, e.g. at oil or air and water interface, the amounts of proteins in the D state are less than 1%. Therefore, the flexibility is hardly detectable by routine optical methods. This is possible to detect by the H–D exchange technique and the protease probe method (Imoto et al., 1976; Ueno and Harrington, 1984). It was suggested in the previous paper (Kato et al., 1985) that there was an apparent relationship between the flexibility and functional properties of proteins. However, there is little information on protein flexibility at oil or air and water interface, and it is desirable to elucidate directly an importance of protein flexibility in foam and emulsion formation. This may be proven by using intramolecularly cross-linked protein inhibiting the flexibility.

Imoto et al. (1973) reported that lysozyme was cross-linked intramolecularly at the specific sites by iodine oxidation. Wold (1961) reported that bovine serum albumin was also intramolecularly cross-linked by the bifunctional reagent. These cross-linked proteins seem to decrease the flexibility.

This paper describes the foaming and emulsifying properties of cross-linked lysozyme and albumin and discusses the importance of protein flexibility in foaming and emulsification.

MATERIALS AND METHODS

The preparation of cross-linked lysozyme was carried out by the method of Imoto et al. (1973). Lysozyme was prepared from fresh egg white by a direct crystallization method and recrystallized five times. Lysozyme, 200 mg/10 mL of water, was reacted for 4 h at room temperature with 0.6 mol of I_2/mol of protein added in five portions of 0.04 M I_2 /.48 M KI solution. The pH was kept at 5.5 during reaction by the addition of NaOH solution. Reaction of each portion of triiodine solution was complete before addition of the next, as judged by base uptake and disappearance of iodine color. During this reaction, the oxindole C-2 of tryptophan 108 forms an ester cross-link between the carboxyl group of glutamic acid 35. The products were dialyzed against distilled water and lyophilized. The lyophilized products were purified by ion-exchange chromatography on a column (1.7 × 100 cm) of Bio-Rex 70, equilibrated with 0.013 M sodium borate/0.037 M sodium carbonate buffer, pH 10.0, and eluted with a linear gradient over 2 L to 0.08 M NaCl in the same buffer. The slowest peak of three major peaks was the lysozyme cross-linked between Glu-35 and Trp-108. This peak was

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