Lysine Level in Solvent Fractions of Pearl Millet

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A typical whole grain sample of normal pearl millet was separated into five fractions by the Landry–Moureaux method. The distribution of proteins among the five fractions resembled that found in corn and not that in sorghum. Complete amino acid analysis of the fractions was made. Of special interest was the low level of lysine in fraction V, which is similar to that found in sorghum. On the basis of this finding, it is predicted that high lysine pearl millet when identified will resemble high lysine sorghum, not *opaque-2* corn, in its total lysine content.

Corn (*Zea mays* L.), sorghum (*sorghum bicolor*) (L.) (Moench), and pearl millet (*Pennisetum typhoides*) (*Burm.*) (Stapf and E. C. Hubbard) contribute substantial amounts of energy and protein to the diets of poorer classes in many of the developing countries of the subtropical and tropical regions of the world.

The protein quality of these cereals is low because of inadequate levels of lysine in their proteins. This amino acid is the first limiting amino acid in all cereals (Jansen and Howe, 1964). In corn, but not in sorghum and millet, tryptophan is also inadequate. In 1964, Mertz and co-workers showed that the lysine and tryptophan content of corn endosperm could be doubled by the introduction of the *opaque-2* gene (Mertz et al., 1964; Misra et al., 1975, 1976). After this discovery, plant breeders searched for similar mutations in the economically important cereal grains. Two high lysine types of barley were discovered. The first, a naturally occurring mutation from Ethiopia was discovered in 1968 by Munck and co-workers (Munck, 1972). A chemically induced high lysine mutant of barley was also identified by Ingversen and co-workers (Ingversen et al., 1973). A high lysine mutant (hl) that improves protein quality and biological value of grain sorghum was found by Axtell and co-workers (Singh and Axtell, 1973). A chemically induced high lysine mutant of sorghum was also subsequently identified (Mohan, 1975).

The changes in the levels of amino acids in the endosperms of these three high lysine cereals, when compared with their normal counterparts, are similar (Misra et al., 1976; Munck, 1972; Ingversen et al., 1973; and Guiragossian et al., 1978). The lysine is increased 50–100% in the endosperm of all three cereals when compared with their normal counterparts, and the tryptophan is doubled in the endosperm of corn.

Animal tests show that lysine is a limiting amino acid in pearl millet protein. In 35 days, rats on ground whole kernel millet gained an average of only 24 g each, whereas, similar rats gained an average of 132 g each when 0.3% lysine hydrochloride was added to the diet (Howe and Gilfillan, 1970). Both diets contained adequate levels of all nutrients except for those supplied by the proteins of the millet. Lysine is, thus, the most limiting amino acid in millet, and its addition at the level indicated raised the growth response in rats to about 93% of that observed with casein (Howe and Gilfillan, 1970).

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Table I. Nitrogen Distribution in the Landry-Moureaux Fractions of *Pearl* Millet, Corn, and Sorghum Normal Whole Seeds

L.-M. fractions		% of total N: pearl millet	corn[a]	sorghum[b]
I	albumin-globulin	22.3	16.6	10.0
II	true prolamine	41.4	38.6	15.7
III	prolamine-like	6.8	10.1	31.3
IV	glutelin-like	9.3	10.0	4.5
V	true glutelin	20.9	20.2	29.3
total N extracted		100.7	95.5	90.8
% protein in seed		14.3	10.7	13.5

[a] Misra et al. (1973). [b] Jambunathan et al. (1975).

Pearl millet is a small seeded grain resembling sorghum; one would expect to find high lysine mutant forms similar to the high lysine mutant forms of corn or sorghum. We are, at present, screening part of the world collection of pearl millet for high lysine types. The data presented below on a typical sample of normal pearl millet are useful in predicting the changes that could be expected if a high lysine type of pearl millet is found.

MATERIALS AND METHODS

The inbred line of pearl millet selected for analysis was one of 168 lines grown at the Purdue Agronomy Farm in the summer of 1975. It is designated as variety 52731 in the world collection. The whole seeds were finely ground and protein (N × 6.25) was determined by the microKjeldahl procedure. Fractionation of the seed proteins was carried out according to the method of Landry and Moureaux (1970) as described in detail earlier (Misra et al., 1975). The individual fractions were obtained by extracting the finely ground millet successively with 0.5 M NaCl (fraction I), 70% isopropyl alcohol (fraction II), 70% isopropyl alcohol containing 2-mercaptoethanol (fraction III), borate buffer (pH 10) containing 2-mercaptoethanol (fraction IV), and borate buffer (pH 10) containing 2-mercaptoethanol and sodium dodecyl sulfate (fraction V). Amino acid analysis was conducted according to Spackman et al. (1958).

RESULTS AND DISCUSSION

Table I shows the distribution of nitrogen in the five Landry–Moureaux fractions of normal pearl millet seed. For comparison, we have included the distribution ob-

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Table II. Amino Acid Composition of Landry–Moureaux Fractions[a]

	fractions				
	I	II	III	IV	V
Lys	5.9	0.5	0.1	2.2	3.2
His	5.3	1.7	0.8	3.5	4.0
Arg	14.3	0.9	0.5	2.3	6.7
Asp	14.2	6.2	2.4	2.4	7.3
Thr	4.2	3.3	2.1	3.1	3.9
Ser	5.8	5.4	1.9	2.1	3.7
Glu	17.2	22.8	9.2	17.2	12.7
Pro	3.2	8.2	3.9	6.9	8.2
Gly	5.6	0.7	0.9	3.0	2.9
Ala	7.0	8.1	4.3	3.4	5.2
Cys	2.0	0.8	0.1	0.5	0.3
Val	4.1	4.2	2.4	2.3	4.9
Met	0.7	1.0	0.2	1.1	1.8
Ile	3.8	5.1	2.9	2.3	4.6
Leu	5.5	14.1	5.3	4.2	6.6
Tyr	5.1	2.7	0.6	4.3	4.3
Phe	5.1	7.6	3.4	1.6	6.3
Trp[b]	2.6	1.2	<0.1	<0.1	1.4
ammonia	3.5	4.2	9.9	3.5	1.7
recov. of aa (%)	111.6	94.5	41.0	62.4	88.0

[a] Grams of amino acid/100 g of protein (N × 6.25). [b] Determined colorimetrically (Villegas and Mertz, 1971).

served for normal whole sorghum and corn seed. It can be seen that fraction II, the true prolamine fraction of millet, makes up a much larger percent of the nitrogen than in sorghum. Millet, thus, resembles corn with respect to levels of this fraction. The level of fraction I (albumin and globulins) is higher and the level of fraction V (true glutelin) is lower than that observed with normal sorghum (Table I). Again, fraction III of millet resembles corn rather than sorghum. Sorghum is unique among these three cereals in its high level of this prolamine-like fraction.

Table II shows the amino acid composition of the five Landry–Moureaux fractions of normal pearl millet. Fraction III values are too low because of oxidative deamination during hydrolysis. This is indicated by the high free ammonia value (9.9%). Losses of cystine and methionine also occurred in all fractions. The other four fractions can be compared with the equivalent fractions from normal and high lysine corn endosperm (Misra et al., 1976) and from normal and high lysine sorghum endosperm (Guiragossian et al., 1978). The germ present in the whole cereal of pearl millet probably contributes as in corn (Mertz et al., 1958), primarily to fraction I without changing its amino acid pattern markedly. In corn and sorghum, the whole germ has an amino acid pattern very similar to that of fraction I of corn and sorghum endosperm (see Mertz et al., 1966; Misra et al., 1976; Guiragossian et al., 1978). Therefore, comparisons of millet whole kernel fractions with endosperm fractions of corn and sorghum appear to be justified.

Of special interest to the nutritionist is the level of lysine in these fractions (Table III). The level in pearl millet fractions I–IV appears to resemble that in sorghum and corn. The lysine level of fraction I falls in the range observed for normal and high lysine corn and sorghum endosperm fraction I (4.3–6.3%). Similarly, the lysine levels in fractions II, III, and IV of millet fall in or near the ranges observed for the same fractions of the other two cereals (0.1–0.5, 0.1–0.8, 0.9–2.9, respectively, for fractions II, III, and IV) [see Misra et al. (1976) and Guiragossian et al. (1978)].

Fraction V (true glutelin) in corn endosperm is unique because of its exceptionally high level of lysine (5.7–7.0%) in both normal and high lysine endosperms (Misra et al., 1976). Fraction V of the millet variety contains 3.2% lysine

Table III. Lysine Levels in Landry–Moureaux Fractions of *Pearl* Millet, Corn, and Sorghum

L.-M. fractions		grams/100 g of protein		
		pearl millet	corn[a]	sorghum[b]
I	albumin–globulin	5.9	4.3–6.3	3.2–4.8
II	true prolamine	0.5	0.1–0.5	0.1–0.3
III	prolamine-like	0.1	0.4–0.8	0.1–0.2
IV	glutelin-like	2.2	1.4–2.9	0.9–1.9
V	true glutelin	3.2	5.7–7.0	1.6–2.8

[a] Misra et al. (1976). [b] Guiragossian et al. (1978).

which more closely resembles normal sorghum endosperm fraction V (1.6% lysine) and high lysine sorghum endosperm (2.8% lysine) (Guiragossian et al., 1978). This would suggest that high lysine millet, assuming increased levels of fraction I and V and decreased levels of fraction II as in high lysine corn and sorghum, would resemble high lysine sorghum and not *opaque-2* corn. Since the pearl millet variety studied contains 2.5% (whole seed), its high lysine counterpart, if similar to high lysine sorghum, should contain approximately 50% more lysine or 3.7% lysine. Samples of open-pollinated, Mexican lines of *opaque-2* corn ranged from 4.4 to 4.7% lysine (normal counterparts 2.7–3.0) in analyses made in our laboratories several years ago (Mertz et al., 1975). High lysine sorghum varieties contain 3–3.3% lysine, normal varieties 2.0–2.4% (Singh and Axtell, 1973; Mohan, 1975; Guiragossian et al., 1978). Although high lysine millet would probably not carry the levels of lysine observed in *opaque-2* corn, it would still be superior in protein quality to our current normal varieties of corn, sorghum, and millets and, thus, make a substantial contribution to the diets of humans in the less developed countries where pearl millets are now grown.

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Interactions of Flavor Compounds in Model Food Systems Using Benzyl Alcohol as an Example

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The interaction between [^{14}C]benzyl alcohol and denatured bovine serum albumin or Soyamin 90, chosen as models for food proteins, was shown to be reversible adsorption of the flavor compound on the denatured protein. Adsorption is directly proportional to the amount of protein and increases linearly with increasing flavor concentration. Adsorption can be decreased slightly by suspending the denatured protein in media containing dissolved protein or lipid material. Adsorption is reduced by 50% when the denatured protein is suspended in media containing both dissolved protein and lipid material, such as caseinate-stabilized oil/water emulsions, aqueous coffee whiteners, or milk. Adsorption isotherms are given for these and other model systems.

Flavor compounds often interact with food components so that their potential is decreased and the desired flavor is altered. The mechanisms for these interactions, especially when proteins are involved, are not completely understood. Maintaining an acceptable flavor in synthetic protein-enriched foods, or those prepared from alternative protein sources, has proven especially challenging to the flavor chemist.

Usually the interaction of flavor compounds with proteins has been investigated by means of headspace analysis. Franzen and Kinsella (1974) used model systems to establish parameters affecting the binding of volatile flavor compounds. They concluded that the addition of protein decreased the concentration of headspace volatiles above aqueous systems. The proteins studied showed no consistent or predictable effect with respect to the magnitude of flavor binding, however, and no one flavor was preferentially bound by all proteins.

Comparing results from headspace analysis, which is a gas–liquid system at equilibrium, with those obtained from a high vacuum transfer method, where equilibrium between bound and free volatiles is constantly disturbed, Gremli (1974) could comment on the nature of binding. He concluded that the absence of ketone retention under the second treatment indicated that ketones were reversibly bound, whereas the partial retention of the aldehydes under high vacuum transfer would suggest both reversible and irreversible interactions. Other experiments showed that an equilibrium exists between bound and free volatile which is independent of the amount of volatile added. In other words, the protein is not saturated even at high dosages. It is difficult to explain the interactions between flavor compounds and food proteins (usually denatured) in terms of classical binding. Gubler and co-workers (1974) reported that the interactions of citral and food ingredients were independent of pH and probably not of ionic character. King (1978) obtained adsorption isotherms for denatured proteins suspended in buffer solutions of flavor compounds. She showed that adsorption was independent of pH and ionic strength over a range of 4.5 to 7.4 and 0.01 to 0.40, respectively, as well as independent of temperature between 7 and 40 °C.

This paper discusses a simple method for direct measurement of interactions between flavor compounds and denatured proteins. Benzyl alcohol is used in model systems to show the nature and extent of these interactions as well as how they can be influenced.

EXPERIMENTAL SECTION

Apparatus. An Isocap 3000 counter from Nuclear-Chicago was used for liquid scintillation counting. Radioactivity of samples was counted in a complete xylene–surfactant based Phase Combining System obtained from The Radiochemical Centre (GB-Amersham).

Protein preparations were lyophilized or dried in either a Sauter vacuum oven (20 h, 62 °C, 70 cmHg), or in a Bühler air drier (18 h, 60 °C). A Fritsch pulverisette, Type P-0150, was used to grind dried protein until it passed through a 0.125-mm sieve.

Protein. Soyamin 90, a commercial preparation of denatured, dried soy protein commonly used in the food industry, was supplied by Lucas Meyer Co. (D-Hamburg). It was dialyzed against distilled water and freeze-dried before use. Bovine serum albumin (BSA) was obtained from Povite Production N.V. (NL-Amsterdam) and heat denatured at the isoelectric point for 25 h at 60 °C. For some experiments fatty acids were removed from BSA before denaturation by treating the native protein with activated charcoal (Chen, 1967).

Flavor Solutions. ^{14}C-labeled benzyl alcohol was obtained from The Radiochemical Centre (GB-Amersham). Various dilutions of unlabeled benzyl alcohol were made in volumetric flasks and spiked with a given small amount of the ^{14}C-labeled compound. The amount was maintained sufficiently small so that the contribution of the radioactive ligand to the initial ligand concentration could be neglected.

Benzyl alcohol was dissolved directly in the medium used to suspend the denatured protein. The following were used as media: 1/15 M Sørensen phosphate buffer pH 6.0; aqueous solutions of 2% casein hydrolysate or 5% sodium caseinate; oil/water (O/W) emulsions prepared with com-

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