NOTE

NUTRITION

Effect of Extrusion on Sorghum Kafirin Solubility[1]

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In young children, apparent protein digestibility of boiled porridge made from whole grain sorghum (tannin-free) was low when compared to that of other cereals (46% vs. 81 and 73% for wheat and maize, respectively) (MacLean et al 1981). However, sorghum that was decorticated, extruded (high temperature, low moisture), and boiled to make a porridge had digestibility markedly increased to 81%. Also, the recovered energy in the feces decreased from 21% of dietary intake to 9% (MacLean et al 1983). Sorghum and, to a lesser extent, rice proteins are unique among the cereal proteins in that they become less digestible after cooking (Axtell et al 1981, Eggum et al 1977, Eggum et al 1983). In contrast to unprocessed sorghum, in which in vitro protein digestibility decreased ~20% due to cooking, the extruded sorghum product showed no decrease when cooked (Mertz et al 1984). In vitro digestibility was 79% for cooked decorticated extruded sorghum, compared to 57% for porridge prepared from plain decorticated flour of the same sorghum cultivar. Sorghum proteins, like maize proteins, can be classified using the Landry-Moureaux (L-M) fractionation scheme based on relative differences in solubility of the different kernel proteins. The procedure involves a sequential extraction of albumins and globulins, kafirins (without and with reducing agent), and glutelins. Two classes of kafirins, which are the storage prolamins of sorghum, are found with this method; they comprise ~55% of total kernel protein (Paulis and Wall 1979). More refined ways of classifying sorghum proteins have recently evolved, such as the classification of kafirins into α, β, and γ types based on differences in solubility, molecular weight, and amino acid composition (Shull et al 1991). However, L-M fractionation of sorghum proteins may still be useful because sorghum has an unusual prolamin profile compared to that of other cereals (Guiragossian et al 1978). A majority of kafirins are soluble in L-M fraction III, which requires the addition of a reducing agent to aqueous alcohol to break disulfide linkages. This may relate to digestibility, because the addition of a reducing agent substantially increases digestibility of cooked sorghum (Hamaker et al 1987). In this note, L-M protein patterns of decorticated extruded sorghum, decorticated sorghum, and whole grain maize and millet are compared.

MATERIALS AND METHODS

Sorghum (cv. 954063) was grown at the Purdue agronomy farm and decorticated at the Carlsberg Research Laboratory, Copenhagen, Denmark. The flour was then extruded at Colorado State University using a Brady extruder (also called a Brady crop cooker). This is a single-screw device with a conical annulus die that produces slightly expanded flakes and fines (Harper and Jansen 1985). This procedure processed low-moisture flour (<20% mc) at 177°C with a throughput of 345 kg/hr. The decorticated, extruded flour used in this study was sampled from the same lot used in the metabolic balance studies, which showed high apparent protein digestibility of 81% (MacLean et al 1983).

In vitro protein digestibility (pepsin) of the decorticated raw and extruded sorghum flours was determined by the method described by Mertz et al (1984). This method simulates the digestibilities found in human studies of sorghum processed by various methods. Raw and extruded flours were cooked (1:10 ratio of flour to water) in a boiling water bath for 20 min to a porridge or thick paste consistency, mixed with a buffer containing pepsin, and digested for 2 hr at 37°C. This further cooking of the extruded flour followed the procedure used in the metabolic study of MacLean et al (1983). Nitrogen in the flour and indigestible residue were determined by micro Kjeldahl and percent digestibility calculated.

L-M protein fractionation (Landry and Moureaux 1970) was performed on the two sorghum flours as described by Guiragossian et al (1978), except that *t*-butanol was used instead of isopropanol for a more complete extraction of the kafirins (Taylor et al 1984). Albumins and globulins (fraction I) were first extracted in 0.5*M* NaCl, and sequentially, prolamins (kafirins) were extracted in 60% *t*-butanol, and the same plus 0.5% 2-mercaptoethanol (2-ME) (fractions II and III). The two classes of kafirins have been designated as kafirin-1 and -2. The second fraction also has been called cross-linked kafirin (Guiragossian et al 1978). Fraction IV (glutelin-like protein) was extracted in an alkali borate buffer plus 0.5% 2-ME. True glutelins (fraction V) were extracted in the same buffer plus 2% sodium dodecyl sulfate. Nitrogen was determined by the micro Kjeldahl method.

Fractions II and III were prepared for amino acid analysis by filtration of the extracts through glass wool, extensive dialyzation against purified water at 4°C, and lyophilization. Lyophilized materials containing ~1-2 mg of protein were hydrolyzed with 6*N* HCl (containing the internal standard norleucine) in a sealed vacuum tube at 110°C for 24 hr. Hydrolyzates were dried under vacuum and twice resuspended in water and dried. Samples were suspended in citrate buffer (pH 2.2), filtered, and injected into an amino acid analyzer (Beckman 119 CL, Palo Alto, CA) for quantification.

RESULTS AND DISCUSSION

Pepsin digestibility of porridge made from the decorticated, extruded product was 18% higher than that of porridge made from raw decorticated flour (Table I); this concurs with the findings of Mertz et al (1984). Previously reported values (using the same digestibility method for whole grain sorghum) were 81% for uncooked flour and 65% for cooked porridge (Hamaker et al 1986). The extrusion process appeared to keep digestibility at the level of uncooked flour, preventing the decrease normally observed upon cooking boiled porridge. Protein digestibility of the extruded sorghum was found to be in the same range as maize in a human metabolic study (MacLean et al 1983). Millet, a cereal closely related to sorghum, is also well digested in vitro.

Solubility properties of the sorghum kafirins were affected by the extrusion process (Table I). The kafirins in the extruded material were mostly soluble in aqueous alcohol (32% of total protein, L-M fraction II), compared to the majority of kafirins in untreated sorghum that required aqueous alcohol plus reducing agent for solubilization (33% of total protein, L-M fraction III). The shift in kafirin solubility in the extruded sorghum produced a ratio between the two kafirin fractions that was similar to that

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found in maize and millet, both of which are relatively well digested. In extruded sorghum, there was also an unexplained shift in glutelin protein (fraction V) to the nonextractable fraction, which evidently did not affect its digestibility.

Although it is not clear on the biochemical level why sorghum contains a high amount of kafirin-2 (L-M fraction III) relative to other cereals, recent work in the area of sorghum protein chemistry and endosperm microstructure may explain how the high level of this fraction could be related to digestibility. Kafirins can be classified on the basis of amino acid composition, molecular weight, and solubility into α-, β-, and γ-, kafirins (Shull et al 1991), which, in vitreous endosperm, comprise 80–84%, 7–8%, and 9–12% of total kafirin, respectively (Watterson et al 1993). Shull et al (1992) recently showed that the high cystine γ-kafirin is found at the periphery of the protein body, whereas the central portion is composed mostly of α-kafirin. Kafirins are the last protein group in sorghum to be digested (Bach Knudsen and Munck 1985, Hamaker et al 1986). This is likely related to the fact that they are found encapsulated in protein bodies. A recent finding in our laboratory showed that isolated unreduced kafirin-1 (which is 94% α-kafirin) was well digested both uncooked and cooked (B. R. Hamaker, *unpublished data*). This suggests that α-kafirin is easily digested, and that access of digestive enzymes to the central portion of the protein body is probably restricted by other hard-to-digest disulfide-bonded proteins, such as γ-kafirin. Such disulfide-bound proteins may also prevent the extraction of much of the α-kafirin in *t*-butanol, thereby producing the large L-M fraction III in sorghum solubilized only with reducing agent. We speculate that the extrusion process disrupted the structure of the protein bodies due to the heat and shearing action involved in the process. Thus, it permitted easy access to α-kafirin both by digestive enzymes and *t*-butanol. This would result in the observed higher L-M fraction II (kafirin-1) and higher digestibility of the extruded material.

Amino acid analysis of L-M fractions II and III of the decorticated raw and extruded material showed little, if any, change in the amino acid profiles due to the shift in fraction amounts (Table II). In all four cases, the compositions were closest to the α-kafirin profile. That is not surprising, because recent findings showed that α-kafirin comprises up to 80% of total kafirin, and the majority of γ-kafirin was found in the glutelin fractions (B. R. Hamaker, *unpublished data*). The increase in kafirin-1 (L-M fraction II) in the extruded material appears to be due to an increase in α-kafirin solubility.

TABLE I
Pepsin Digestibility of the Cooked Flour Porridges of Decorticated Sorghum, Decorticated Extruded Sorghum, Maize, and Millet; and Landry-Moureaux (L-M) Fractions of the Four Flours

	Decorticated Sorghum	Decorticated Extruded Sorghum	Whole Maize[a]	Whole Millet[b]
Pepsin Digestibility (%)	61 (2.1)[c]	79 (1.3)	82	86
L-M Fraction (% of total N)				
I Albumin and globulin	8 (0.1)	6 (0.4)	20	28
II Prolamin-1	20 (0.6)	32 (0.9)	34	39
III Prolamin-2	33 (0.4)	16 (0.9)	10	3
IV Glutenlin-like	Tr[d]	Tr	10	7
V Glutelin	20 (0.3)	5 (0.2)	16	14
Nonextractable[e]	14	41	10	9

[a] From Hamaker et al (1986).
[b] From Ejeta et al (1987).
[c] Mean (standard deviation in parenthesis).
[d] Trace (<0.1%).
[e] Determined by difference from 100%.

TABLE II
Amino Acid Composition (mol %)[a] of Landry-Moureaux (L-M) Fractions II and III for Raw and Extruded Decorticated Sorghum, and of α-, β-, and γ-kafirins

Amino Acid	L-M II[b]		L-M III[b]		Kafirin[c]		
	Raw	Extruded	Raw	Extruded	α	β	γ
Asx	6.1	6.0	5.4	5.0	5.7	3.5	0.7
Thr	2.7	2.2	2.3	2.5	3.3	4.9	4.5
Ser	4.3	4.7	5.3	5.2	5.9	4.9	5.4
Glx	21.9	23.7	23.2	23.4	22.2	18.8	14.9
Pro	9.8	10.4	10.6	10.3	9.1	10.3	24.4
Gly	1.8	1.6	2.1	2.3	4.2	7.2	9.3
Ala	14.4	14.2	14.2	14.3	15.5	14.2	6.8
Val	4.8	4.5	4.2	4.4	4.9	5.5	6.3
Met	1.0	0.5	1.1	1.1	0.6	6.0	1.1
Ile	3.8	3.7	3.6	3.6	4.5	2.4	2.8
Leu	18.6	18.3	17.6	17.7	15.4	12.7	9.7
Tyr	3.6	3.5	3.7	3.7	2.3	3.2	2.3
Phe	5.0	5.2	5.1	5.1	4.1	2.0	1.9
His	1.0	1.0	0.9	0.9	1.2	1.0	7.4
Lys	0.1	0.1	0.0	0.0	0.4	0.5	0.4
Arg	1.1	1.0	0.8	1.1	0.8	2.9	2.2

[a] Calculated minus cysteine and tryptophan.
[b] Coefficient of variation was less than 5% for each amino acid.
[c] From Shull et al (1992) for sorghum cultivar P721 N.

CONCLUSIONS

Although the shift in L-M kafirin fractions in extruded sorghum does not, in itself, explain why in vitro and in vivo protein digestibility increased after extrusion, it points out an interesting potential relationship between kafirin solubility and digestibility. Maize is a member of the same tribe as sorghum, *Andropogoneae,* and its prolamins are very similar to those of sorghum (similar amino acid composition, structure, and location; high degree of sequence homology; and antibody cross-reactivity), yet maize is relatively well digested, and its digestibility does not decrease upon cooking. The solubility difference between sorghum and maize is one of the few readily apparent differences in their proteins. In this study, extruded sorghum protein digestibility was increased with a concomitant change in L-M prolamin classes to a ratio similar to that found in maize and millet. Other sorghum cultivars should be tested to verify this finding. Further studies on why kafirin solubility in the unprocessed grain differs from maize and millet may be useful in determining the underlying reason why sorghum is less digestible.

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