Protein quality in cereals and pulses

2. Influence of polyethyleneglycol on the nutritional availability of methionine in sorghum (*Sorghum vulgare* Pers.), field beans (*Vicia faba* L.) and barley

BY J. E. FORD AND D. HEWITT

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(*Received* 23 *October* 1978 – *Accepted* 28 *March* 1979)

1. Polyethyleneglycol (PEG 4000) was examined for its influence on relative nutritional value (RNV) and available methionine in sorghum (*Sorghum vulgare* Pers.), field beans (*Vicia faba* L.) and barley, as measured microbiologically with *Streptococcus zymogenes*. The results were assessed in relation to the content of tannins in the test samples.

2. In grain of hybrid sorghum the RNV averaged 87 (range 79–92) for six low-tannin varieties and 41 (30–53) for eleven high-tannin varieties. The corresponding available methionine values averaged 17·0 (15·7–18·9) and 8·9 (6·7–11·0) g/kg protein. Addition of PEG 4000 to the test samples increased the average RNV of the high-tannin varieties from 41 to 78, and the average available methionine content from 8·9 to 16·2 g/kg protein.

3. With seed of ten coloured-flowered varieties of field beans, treatment with PEG gave a small but consistent increase in the available methionine content, which resulted from the inactivation of tannins in the testa.

4. In twenty-three samples of barley grain, treatment with PEG had no effect on the values obtained for available methionine.

5. Treatment of high-tannin sorghum grain with ammonia has been reported to inactivate the tannins and increase the nutritional value for rats and chicks. This finding was confirmed. The present study showed that ammonia and PEG 4000 were equally effective in enhancing the nutritional quality as measured in the microbiological tests.

Tannins are present in many food crops and they may significantly impair the nutritional quality. Thus, for example, in some varieties of field beans (*Vicia faba* L.) the seeds are rich in condensed tannins, and their inclusion in the diet of single-stomached animals has been reported to depress the apparent digestibility of the protein (cf. Ronnenkamp, 1977; Martin-Tanguy *et al.* 1977). Similarly in sorghum grain (*Sorghum vulgare* Pers.), the protein quality in some genotypes is severely limited by the high level of tannins in the testa (Axtell *et al.* 1975). In both species tannin content is simply inherited and controlled by only one or two major genes, and it is no difficult matter to eliminate the tannin factors. A question then arises, whether in selecting for low tannin content the breeder may lose important agronomic advantages of the high-tannin lines. In sorghums, the high-tannin varieties are less susceptible to depredation by birds and are more resistant to preharvest germination and mould growth (Harris, 1969; Harris & Burns, 1973), and the tannins may serve in a similar protective role in other plant species. In this situation, a practical alternative to the development of low-tannin varieties might be to remove or inactivate the tannins in existing high-tannin varieties. With sorghum there are several ways in which this can be done. The tannins can be removed by mechanical de-hulling, though with considerable loss of protein (Chibber *et al.* 1978). Alternatively, they can be removed by treatment of the grain with sodium hydroxide solution (200 g/l) at 71° for 6 min (Blessin *et al.* 1971), or with concentrated ammonia solution at room temperature for 7 d (Price & Butler, 1978). This latter treatment greatly improved the nutritional quality of a high-tannin sorghum, but it depressed that of a low-tannin variety. Both these 'chemical' treatments would need to be carefully controlled or they might do more harm than good. Alkali treatment of vegetable proteins can severely

0007-1145/79/3239-2803 $01.00

depress the nutritional quality (Provansal *et al.* 1975) and it is a question of topical interest whether lysinoalanine and other unnatural amino acid derivatives formed during heat treatment of protein at high pH may be harmful to health (Friedman, 1977).

Another approach is to inactivate the tannins by the addition of adsorbents to which they bind more strongly than to protein. Polyvinylpyrollidone (PVP) and several derivatives of polyethyleneglycol (PEG) have been used for this purpose in the extraction of enzymes from higher plants (Jones & Hulme, 1961; Loomis & Battaile, 1964; Boudet, 1965; Jones, 1965), and Rayudu *et al.* (1970) showed that this property of binding tannins is operative in vivo. They found that PVP and Tween 80 (polyoxyethylene sorbitan mono-oleate) markedly reduced the growth-depressing effect of tannic acid in chicks, though at the levels tested neither compound restored growth to equal that in control birds given a tannic acid-free diet. Ford (1977) examined the effects of PEG, PVP, Tween 80 and Lissapol NDB (a non-ionic detergent containing PEG with substituent phenyl groups) on the nutritional availability of methionine in a high-tannin sorghum. All increased the availability coefficient (i.e. the ratio, available:total) from 0·46 to approximately 0·95, as measured microbiologically with *Streptococcus zymogenes*. And in biological tests on the same sorghum, supplementation of the test diet with 0·1 g PEG 4000/g protein increased the true digestibility of the nitrogen for chicks from 0·44 to 0·90, and for rats from 0·53 to 0·92 (Ford & Hewitt, 1977).

The present paper reports further in vitro experiments on the influence of PEG on the availability of methionine in the selection of sorghums, field beans and barleys described in the preceding paper (Ford & Hewitt, 1979 *b*), and an experiment to test the efficacy of treatment of sorghum grain with ammonia (cf. Price & Butler, 1978) as an alternative means of inactivating the tannins.

EXPERIMENTAL

Seed was examined of nineteen varieties of hybrid sorghum, twenty-three of barley and twenty of field beans and, in addition, a preparation of testa from seeds of the high-tannin field bean variety Beagle. The test samples are described by Ford & Hewitt (1979*a*), as are the methods used in the assay of total and available methionine and of tannins.

Influence of PEG. The effect of addition of PEG on the availability of methionine was measured as follows. Two portions of each test sample were weighed out into screw-stoppered 25 ml bottles, in amounts calculated to contain 12·5 mg N, and suspended in 10 ml sodium β glycerophosphate solution (20 g/l). To one was added 100 mg PEG 4000 (BDH Chemicals Ltd). The pH value of both was then adjusted to 8·0 and 1 ml pronase (B grade; Calbiochem Ltd) (10 g/l in the glycerophosphate solution) was added. The mixture was incubated at 48° for 3 h in an 'end-over-end' shaker, and then adjusted to pH 7·2 and diluted with water to contain 5–10 μg methionine/ml.

Treatment of sorghum grain with ammonia solution. The procedure was modelled on that described by Price & Butler (1978), but using only 320 g sorghum grain (cf. 32 kg). Thus, to 320 g whole grain in a 500 ml screw-stoppered polythene bottle was added 18 g ammonia solution (approximately 350 g NH_3/kg). The mixture was shaken and allowed to stand at room temperature for 24 h. A further 18 g ammonia solution was then added and the mixture allowed to stand, with occasional shaking. On the seventh day the grain was spread on a stainless-steel tray and dried overnight in a forced-draught air oven at 30°. The grain was then milled to pass 40 mesh, whereupon a strong smell of ammonia was detected. It was dispelled by further airing of the meal at 30°.

Amino acid analysis. The amino acid composition of some of the sorghum samples was determined with an LKB automatic amino acid analyser (LKB Ltd). Samples containing 2 mg N were hydrolysed at 110° for 2 h with 10 ml 6 M-hydrochloric acid, under N_2 in a sealed

tube. The standard amino acid mixture was supplemented with a reference preparation of lysinoalanine (Miles Laboratories Ltd).

Statistical analysis. Most of the assays were done in duplicate, and the data subjected to analysis of variance. The effect of PEG and the 'PEG × variety' interaction were tested for significance against the 'test material × assay' interaction mean square. Mean values are given, and least significant differences between test materials based on this interaction mean square.

RESULTS AND DISCUSSION

Experiments with sorghum. Table 1 sets out results for seventeen varieties of sorghum, listed in order of increasing tannins content. The RNV values, determined after predigestion of the test samples with pronase and in absence of PEG, fell into two distinct groups, averaging 86·8 (range 79–92) for the low-tannin varieties nos. 1–6 and 41·0 (range 30–53) for the intermediate- and high-tannin varieties nos. 7–17. The same was true for the available methionine values, and for the ratio, available:total methionine, which averaged 1·03 (0·85–1·14) for the low-tannin varieties and 0·52 (0·38–0·65) for the remaining varieties.

The total methionine assays were corrected for mean recovery of L-methionine added to the test samples before hydrolysis (Ford & Hewitt, 1979*a*). Differences between varieties were small as compared with those for available methionine, and they were not related to the tannins content nor to the wide differences in protein nutritional quality that were so evident in the RNV ratings.

Addition of PEG 4000 to the test samples before assay changed this picture. The average RNV for the low-tannin varieties increased only marginally, from 86·8 to 88·8 ($P > 0{\cdot}05$), but that for the intermediate- and high-tannin varieties increased nearly twofold, from 41·0 to 78·0. For each of these latter varieties the increase was significant ($P < 0{\cdot}001$), though the magnitude of the increase varied ($P < 0{\cdot}001$). The effects of PEG on available methionine (g/kg protein) paralleled those on RNV, and again for the tannin-containing varieties (nos. 7–17) the magnitude of the increase varied between varieties ($P < 0{\cdot}05$). The average value for available:total methionine in these latter varieties also increased sharply, from 0·52 to 0·95.

The culture medium used in the microbiological assays contains Tween 80, which is added to prevent non-specific stimulation of growth by oleic and other fatty acids in the test extracts. Previous work (Ford, 1977) had shown Tween 80 to be fully as effective as PEG 4000 in improving the nutritional availability of methionine in high-tannin sorghum grain, and so its presence at a concentration of 1 g/l in the assay medium might have enhanced the results for the high-tannin sorghums. To check on this the samples were assayed again, with and without added PEG 4000, using culture medium from which the Tween 80 had been omitted. There was marked non-parallelism between standard and test growth responses, and upward drift in results calculated for increasing dose levels. The assay was clearly not valid, but the results (Table 1) were reassuring in that they showed much the same 'spread', and the same response to PEG 4000, as those obtained with the complete medium.

Table 2 compares the effects of ammonia and PEG 4000 in the pretreatment of yellow (low-tannin) and brown (high-tannin) sorghum grain. With the yellow sorghum neither treatment had any effect on the RNV or on the high availability of methionine. With the brown sorghum the available:total methionine value (0·51) and the RNV (43) were comparatively very low, but they increased to equal those for the yellow sorghum after treatment of the grain with ammonia or PEG 4000. The results are from single assays, but they are closely consistent with those from other tests in which BR 54 (high-tannin) and RS 690 (low-tannin) were treated for 4 d and 7 d with one-tenth the concentration of ammonia.

Treatment of high-tannin sorghums with ammonia destroyed most of their tannin activity

Table 1. *Influence of polyethyleneglycol (PEG 4000) on the relative nutritional value (RNV)* and availability of the methionine* of grain of seventeen varieties of sorghum* (Sorghum vulgare *Pers.*), *in relation to the tannins content*

(Values in parentheses represent the 'available' values as a proportion of the corresponding 'total')

				Methionine (g/kg protein (nitrogen × 6·25))				
		RNV			Available			
Sorghum variety	Tannins index†	−PEG 4000	+PEG 4000	Total	−PEG 4000	+PEG 4000	−PEG‡ 4000	+PEG‡ 4000
SSK 52	0·22§	83§	83§	13·9§	15·8 (1·14)	16·5 (1·19)	22·4	22·6
E 57	0·24	91	95	16·6	17·3 (1·04)	18·0 (1·08)	25·3	26·4
ORO 7	0·28	92	96	18·4	18·9 (1·03)	20·1 (1·09)	25·8	27·7
TE 66	0·29	79	84	18·5	15·7 (0·85)	16·6 (0·90)	19·3	19·5
RS 671	0·34§	86§	86§	15·8§	15·7 (0·99)	15·9 (1·01)	19·5	19·8
74–115	0·41§	90§	89§	16·6§	18·4 (1·11)	18·6 (1·12)	23·2	23·5
RS 617	0·96	44	79	18·4	9·0 (0·49)	15·8 (0·86)	9·2	18·0
Savannah	1·04	46	82	17·7	9·9 (0·56)	17·5 (0·99)	11·6	22·2
Shoobird	1·08	45	81	18·1	10·4 (0·57)	16·8 (0·93)	10·6	21·9
AKS 663	1·10	53	80	17·0	10·5 (0·62)	15·5 (0·91)	14·5	23·0
Ga 615	1·16	35	71	17·4	8·8 (0·51)	16·0 (0·92)	7·9	19·5
AKS 618	1·22	30	58	16·3	8·1 (0·50)	12·8 (0·79)	6·9	17·2
BR 76	1·23	33	77	17·5	6·7 (0·38)	16·1 (0·92)	7·7	21·4
74–123	1·30§	53§	87§	16·8§	11·0 (0·65)	18·5 (1·10)	15·3	24·0
BR 54	1·80§	38	84	18·0§	8·4 (0·47)	16·0 (0·89)	7·2	17·7
X 3101	1·90	39§	76§	14·5	8·3 (0·57)	15·5 (1·07)	8·8	18·8
74–118	1·90§	35§	83§	16·0§	7·2 (0·45)	17·2 (1·08)	8·5	22·0
LSD (P=0·05)	0·18	5·9		1·3	1·9			

LSD, least singificant difference.
* Measured with *Streptococcus zymogenes* as described by Ford & Hewitt (1979*a*).
† Measured as described by Ford & Hewitt (1979*a*).
‡ Tween 80 omitted from culture medium (see p. 319).
§ One missing value.

Table 2. *Availability of methionine* and relative nutritional value (RNV*) of low- and high-tannin varieties of sorghum* (Sorghum vulgare *Pers.*), *as influenced by pretreatment of the whole grain with ammonia solution, or of the milled grain with polyethyleneglycol (PEG 4000)*

		Tannin content		Methionine (g/kg protein (nitrogen × 6·25))			
Sorghum variety	Pretreatment	Catechin equivalent†	Tannins index‡	Total	Available	Available / Total	RNV
Yellow (low-tannin)	None	0·25	0·15	16·9	15·6	0·92	87
	Ammonia§	0·15	0·11	—	15·7	0·93	88
	PEG 4000	—	—	—	15·5	0·92	90
	Ammonia + PEG§	—	—	—	16·4	0·97	86
Brown (high-tannin)	None	2·9	1·20	17·0	8·6	0·51	43
	Ammonia§	0·20	0·97	—	15·5	0·91	96
	PEG 4000	—	—	—	15·7	0·92	91
	Ammonia + PEG§	—	—	—	16·3	0·96	92

* Measured with *Streptococcus zymogenes* as described by Ford & Hewitt (1979*a*).
† Measured by the vanillin method of Burns (1971).
‡ Measured as described by Ford & Hewitt (1979*a*).
§ Treatment of the grain with ammonia solution increased the N content of the yellow sorghum by 17·2% and of the brown by 19·6%. This increase was ignored in calculating the results, which are expressed per unit N in the untreated grain.

Table 3. *Influence of polyethyleneglycol (PEG 4000) on results for available methionine* in white- and coloured-flowered varieties of field beans* (Vicia faba *L.*)

((*a*) Mean values for ten white- and ten coloured-flowered varieties; (*b*) individual values for two white- and six coloured-flowered varieties)

		Available methionine (g/kg protein (nitrogen × 6·25))		
		−PEG 4000	+PEG 4000	Change (%)
		(*a*)		
White-flowered varieties				
Mean		6·47	6·48	0·24
Range		5·57–7·23	5·64–7·13	−2·31 to +4·28
Coloured-flowered varieties				
Mean		6·20	6·51	4·95
Range		5·84–6·76	6·11–7·00	−0·59 to +9·42
		(*b*)		
Variety	Tannins index†	−PEG 4000	+PEG 4000	Change (%)
Triple white	0·050	6·90	6·94	+0·5
Compacta	0·014	6·92	6·83	−1·2
Blaze	0·835	5·88	6·27	+6·5
Bead	0·835	5·90	6·18	+4·9
Throws	0·908	5·92	6·28	+6·1
Bulldog	0·710	6·32	6·70	+6·0
Compacta brown	0·710	6·24	6·68	+7·0
Felix	0·688	6·66	7·06	+6·0
LSD ($P=0·05$)			0·64	

LSD, least significant difference.
* Measured with *Streptococcus zymogenes* as described by Ford & Hewitt (1979*a*).
† Measured as described by Ford & Hewitt (1979*a*).

as measured in the vanillin test, but the tannins index showed only a small decrease. It seemed likely that an effect of the ammonia was to condense the tannins and reduce their solubility in the methanol used as the extractant in the vanillin test.

Amino acid analysis showed no trace of lysinoalanine in acid hydrolysates of the ammonia-treated samples, nor any other change in amino acid composition.

Experiments with field beans. Table 3(*a*) gives results for seed of ten white- and ten coloured-flowered varieties, obtained in a single assay. With the 'white' varieties the PEG treatment had little effect on the results (average change +0·24%; range −2·31 to 4·28); with the 'coloured' varieties it gave a small increase (average change +4·95%; range −0·59 to 9·42).

For eight of the samples, two 'white' and six 'coloured', available methionine was measured again in two independent assays (Table 3(*b*)). With all the 'coloured' varieties, PEG treatment gave a small increase, which did not differ significantly between varieties ($P>0·05$), and for the group was significant ($P<0·01$). It resulted apparently from the release of tannin-bound protein from the testa. Thus, for 'coloured' variety Beagle, PEG increased the available methionine value for the testa from 3·9 to 8·7 g/kg protein, but it had no effect on the value for the cotyledons (9·0).

Experiments with barleys. Twenty-three samples of barley grain were twice assayed for

available methionine, each time in presence and in absence of PEG during the enzymic pretreatment. In absence of PEG the mean content of available methionine (g/kg protein) was 17·8 (range 15·7 to 20·4), and in presence of PEG the corresponding value was 18·0 (16·3 to 19·7). The increase with PEG (0·2 ± 0·16) was small and not significant ($P > 0·05$). The test digests were re-assayed using culture medium from which Tween 80 had been omitted, and again the PEG treatment showed no effect.

The mean tannins index value for the barleys was 0·18 (range 0·14 to 0·22) (Ford & Hewitt, 1979*a*), as compared with 0·30 (range 0·22 to 0·41) for six low-tannin sorghums with which PEG treatment gave a small increase in mean available methionine content, from 17·0 to 17·6 g/kg protein. This increase, 0·6 ± 0·38, was not significant ($P > 0·05$).

General

Sorghum constitutes a major proportion of the world food-grain production, but the high tannins content and consequent poor digestibility of many varieties has discouraged their use as a primary food source. The present findings hold promise that use of PEG in the formulation of sorghum diets, or pretreatment of the sorghum grain with ammonia, might remove this nutritional handicap at small cost. But further study is needed to ensure that the procedures have no adverse effects. Certainly the use of ammonia could not be advocated without reservations concerning its possible toxicity. Price & Butler (1978) reported that ammoniation of a low-tannin sorghum depressed the nutritional quality for rats and chicks, though in their experience the treatment did not significantly increase the N content. We found that the N content was increased by 17·2%, even after the treated grain had been ground and exposed to air until no smell of ammonia could be detected (Table 2). Ammonia salts are generally recognized as safe (GRAS; National Technical Information Service, 1973) if ingested in moderate amounts, but large intakes are toxic and may cause acidosis and liver and kidney damage. Visek (1974) warns of several possible dire effects of increasing the content of ammonia within the alimentary tract by addition of non-specific N to the diet.

The tannins are a heterogeneous class of plant phenolics and their anti-nutritional effects vary widely according to their composition and extent of polymerization. In the present study, high-tannin sorghums were much more improved by supplementation with PEG than were field beans of similarly high tannins content as measured by the tannins index procedure, and it would seem that 'tannins index', like other chemical measures of tannins content, is not a reliable pointer to the growth-depressing activity. It is however useful for grading among samples of similar designation.

In the barleys, the lack of response to supplemental PEG was associated with low and uniform tannins content. This indication that the tannins in barley are of little nutritional importance was confirmed in biological tests, as also were the findings for sorghum and field beans (Ford & Hewitt, 1979*b*).

The authors are grateful to Mrs Dinah Bishop for technical assistance, and to the following persons who kindly provided the samples for test: Dr J. D. Axtell, Dr D. Bond, Dr Eva Holmberg, Mr G. C. Mann, Professor T. S. Nelson, Dr C. Shorrock and Dr S. Thomke.

REFERENCES

Axtell, J. D., Oswalt, D. L., Mertz, E. T., Picket, R. C., Jambunathan, R. & Srinivasan, G. (1975). In *High Quality Protein Maize*, pp. 374–386. Stroudsburg, Pa.: Dowden, Hutchinson & Ross.
Blessin, C. W., Anderson, R. A., Deatherage, W. L. & Inglet, G. E. (1971). *Cereal Chem.* **48**, 528.
Boudet, A. (1965). *C. r. hebd. Séanc. Acad. Sci., Paris* **261**, 214.
Burns, R. E. (1971). *Agron. J.* **63**, 511.
Chibber, B. A. K., Mertz, E. T. & Axtell, J. D. (1978). *J. agric. Fd Chem.* **26**, 679.
Ford, J. E. (1977). *Proc. Nutr. Soc.* **36**, 125A.
Ford, J. E. & Hewitt, D. (1977). *Proc. Nutr. Soc.* **36**, 126A.
Ford, J. E. & Hewitt, D. (1979*a*). *Br. J. Nutr.* **41**, 341.
Ford, J. E. & Hewitt, D. (1979*b*). *Br. J. Nutr.* (In the Press).
Friedman, M. (1977). In *Protein Crosslinking: Nutritional and Medical Consequences*. New York: Plenum Press.
Harris, H. B. (1969). *Proc. 24th An. Corn and Sorghum Res. Conf., Chicago*, Ill. **24**, 113.
Harris, H. B. & Burns, R. E. (1973). *Agron. J.* **65**, 957.
Jones, D. E. (1965). *Nature, Lond*, **206**, 299.
Jones, J. D. & Hulme, A. C. (1961). *Nature, Lond.* **191**, 370.
Loomis, W. D. & Battaile, J. (1964). *Plant Physiol.* **39**, Supp., 12.
Martin-Tanguy, J., Guillaume, J. & Kossa, A. (1977). *J. Sci. Fd Agric.* **28**, 757.
National Technical Information Service (1973). *GRAS Food Ingredients-Ammonium Ion*, PB-221, p. 235. Washington, DC: US Department of Commerce.
Price, M. L. & Butler, L. G. (1978). *Nutr. Rep. int.* **17**, 229.
Provansal, M. M. P., Cuq, J.-L. A. & Cheftal, J.-C. (1975). *J. agric. Fd Chem.* **23**, 938.
Rayudu, G. V. N., Kadirvel, R., Vohra, P. & Kratzer, F. H. (1970). *Poultry Sci.* **49**, 1323.
Ronnenkamp, R. R. (1977). The effect of tannins on nutritional quality of dry beans, *Phaseolus vulgaris* L. PhD Thesis, Purdue University, West Lafayette, Ind., USA.
Visek, W. J. (1974). *J. agric. Fd Chem* **22**, 174.

Printed in Great Britain