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Chemical Composition and *In Vitro* Protein Digestibility of Italian Millett (*Setaria italica*)

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ABSTRACT

The grain from 12 cultivars of Italian millet (Setaria italica) *was analysed for proximate composition, soluble sugars and polyphenols. Ash and fibre content are comparable to that of other millets while protein and calcium levels are slightly higher. The overall composition of Italian millet is not very different from other millets.* In vitro *protein digestibility (IVPD) studies showed that it was high with pepsin but low with trypsin. Acid treatment of the flour increased IVPD with trypsin.*

INTRODUCTION

Italian millet (*Setaria italica*) is one of the important minor millet crops of India. It is used in puddings and other cereal delicacies and also eaten cooked like rice or baked as bread. The mature grain of the common cereals consists of carbohydrates, proteins, lipids, mineral salts and water, together with small quantities of vitamins and other substances, some of which are important nutrients in the human diet. In the present investigation different varieties of Italian millet were examined for proximate principles, calcium,

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total soluble sugars, tannins and phenolics. In order to study the digestibility of Italian millet protein, IVPD with pepsin, trypsin and papain were estimated. Pepsin and trypsin, the proteolytic enzymes of the digestive tract, were chosen to assess the nutritional quality of Italian millet. In view of the wide use of papain in the food industry, where it is used to tenderise meat, IVPD with papain was also undertaken.

MATERIAL AND METHODS

Samples

Twelve cultivars of Italian millet (*Setaria italica*) grown in the same season and locality under standard agronomic practices* were obtained from the University of Agricultural Sciences, Bangalore, India. The seeds were dehulled in a McGill sheller and ground in a Bühler grinder to pass through a 40-mesh sieve.

Enzymes

Pepsin (1:3000, B.P.) was from SD's Fine Chemicals, India, and trypsin (2000 E/g) was a product of E. Merck, West Germany.

Proximate nutrient analyses

The husk containing most of the germ and the hull (bran) was weighed and expressed as a percentage. Official methods of the AOAC (1980) were used to determine moisture (AOAC, 14·063), ash (AOAC, 14·064) and fat (AOAC, 14·067). Fibre was estimated in the samples by the buffered acid–detergent fibre procedure of Baker (1977). The crude protein contents (N × 6·25) were determined in a previous study (Monteiro *et al.*, 1982). Carbohydrate was obtained by difference.

Calcium was analysed by standard methods (Bassett *et al.*, 1978). The quantity of total soluble sugars in the 80% ethanol extract of the defatted millet flours was estimated by the phenol–sulphuric acid method (Dubois *et al.*, 1956) using glucose as standard. Tannins were extracted with 1% HCl in methanol and estimated colorimetrically (Burns, 1971) and expressed as catechin equivalents. Total phenolics were determined as chlorogenic acid (Swain & Hillis, 1959).

* Italian millet, a dry land crop, is sown between June and August. Farmyard manure (2500 kg per acre) is incorporated 2–3 weeks before sowing. Fertilizers (12 kg N and 6 kg P_2O_5 per acre) are applied before or at the time of sowing. The crop takes 100 days for harvest and yields 400 to 600 kg of grain per acre. There are no major diseases in the field or pests in the field or during storage.

In vitro protein digestibility (IVPD) of Italian millet

IVPD of Italian millet samples was determined by calculating the difference between the nitrogen in the sample before and after digestion with pepsin (AOAC, 7·048, 1980).

Two hundred milligrams of whole seed flour were incubated with 50 ml of 0·2% pepsin (1:3000, B.P.) in 0·075N HCl for 24 h at 37°C. The digests were filtered (Whatman No. 2) and the residue washed with warm water. Nitrogen in the residue was determined by the micro-Kjeldahl method.

IVPD with trypsin was determined essentially as with pepsin but in 0·1M phosphate buffer, pH 7·6.

The method of Buchanan & Byers (1969) as modified by Nanda *et al.* (1977) was employed to determine IVPD with papain. To the flour (200 mg) in 9·1 ml of 0·1M citrate–phosphate buffer, pH 6·6, 0·5 ml of 2·5% papain solution and 0·5 ml of activator solution (0·5M thioglycollic acid adjusted to pH 6·6), were added and the mixture was incubated for 24 h at 60°C in stoppered tubes. Ten millilitres of 50% TCA was added, the tubes cooled at 5°C and centrifuged for 15 min at 18 000 *g*. Nitrogen in the precipitate was determined as before.

All digestions were performed in triplicate and appropriate blanks were included.

RESULTS AND DISCUSSION

The results of proximate analysis of the millet samples are shown in Table 1. The content of husk in Italian millet varieties ranged between 20·8% and 28·8%. This is comparable with the proportion of husk in the rice grain which averages about 21%. The husk of oats is about 25% by weight on average. The moisture ranged from 5·9% to 12·8%. The mean ash content of Italian millet is 1·38% which is approximately the same as found in other cereals. Fat ranged between 4·7% and 6·3%, which is similar to the fat content of some other millets (Ramanathan *et al.*, 1975). The millet contained more than 4% fat and hence the keeping quality is likely to be poor owing to the auto-oxidation of fats, as in pearl millet (Indira & Naik, 1971). Carbohydrate determined by difference ranged from 65·0% to 75·7%. These results are comparable with the values obtained by Wankhede *et al.* (1979).

The acid detergent fibre (ADF) content of Italian millet had a narrow range of 5·2%–5·9%. The variety K-221-1 had the highest and JNSE-53 the lowest amounts of ADF. The fibre values agree with the values obtained by Ramanathan *et al.* (1975) for other minor millets. Fibre values do not show

TABLE 1
Proximate Analysis of Italian Millet Samples

Variety	*Husk* (%)	*Moisture* (%)	*Ash*[a] (%)	*Fat*[a] (%)	*Acid detergent fibre*[a] (%)	*Crude protein*[a,b] (%)	*Carbohydrate (by difference)*[a] (%)
CO-3	28·8	12·8	1·6	6·3	5·8	14·3	65·0
I.Se-201	24·8	10·6	1·5	5·5	5·8	14·4	68·1
I.Se-358	25·0	7·3	1·4	5·1	5·7	13·4	72·8
I.Se-480	23·2	8·2	1·4	5·5	5·7	13·4	71·4
I.Se-703	20·8	8·0	1·3	5·9	5·3	13·3	71·6
I.Se-709	27·0	7·0	1·3	5·2	5·3	12·6	74·0
JNSE-26	26·8	11·0	1·5	5·0	5·7	13·5	69·1
JNSE-53	28·5	9·3	1·1	5·2	5·2	15·0	69·4
K-221-1	25·7	5·9	1·6	5·2	5·9	12·3	75·0
KHS-1	23·8	8·3	1·4	4·9	5·4	12·2	73·3
SI-76/4	26·9	6·8	1·2	5·2	5·2	11·1	75·7
SI-80/2	22·7	9·7	1·3	4·7	5·5	12·0	72·1
Mean	25·3	8·74	1·38	5·31	5·54	13·1	71·5
SD	2·42	2·00	0·15	0·44	0·25	1·13	3·09
Range	20·8–28·8	5·9–12·8	1·1–1·6	4·7–6·3	5·2–5·9	11·09–15·04	65·0–75·7

[a] Analyses were made in duplicate and results expressed on a dry weight basis.
[b] Values on protein content from literature (Monteiro *et al.*, 1982) included for completeness of data.

TABLE 2
Calcium Levels, Total Soluble Sugars, Tannins and Total Phenols in Italian Millet[a]

Variety	*Total calcium (mg/100 g)*	*Total soluble sugars* (%)	*Tannins* (%)	*Total phenols* (%)
CO-3	80·0	0·48	0·09	0·15
I.Se-201	92·3	0·62	0·08	0·07
I.Se-358	70·6	0·69	0·09	0·07
I.Se-480	68·8	0·63	0·09	0·10
I.Se-703	73·2	0·59	0·10	0·09
I.Se-709	88·0	0·54	0·09	0·09
JNSE-26	77·8	0·65	0·09	0·09
JNSE-53	85·8	0·59	0·09	0·11
K-221-1	67·5	0·43	0·08	0·08
KHS-1	85·5	0·42	0·09	0·09
SI-76/4	84·7	0·80	0·09	0·11
SI-80/2	88·6	0·47	0·05	0·10
Mean	80·2	0·58	0·09	0·10
SD	8·51	0·11	0·01	0·02
Range	67·5–92·3	0·42–0·69	0·05–0·10	0·07–0·15

[a] Analyses were made in duplicate and results expressed on a dry weight basis.

the true percentage of the food that is unavailable to man. Even though fibre values determined by the usual methods have no bearing at all on the physiology of digestion, these values may still be useful. The difference between fibre and indigestible residue may be taken as an estimte of that part of fibre that is more or less fermented in the large intestine.

Data on calcium content, total soluble sugars, tannin and total phenolics of the 12 varieties of the millet are summarised in Table 2. The calcium content ranged from 67·5 to 92·3 mg/100 g. Compared to finger millet (*Eleusine coracana*), Italian millet is low in calcium (Pore & Magar, 1977). However, Italian millet has a higher calcium content compared to other millet grains (Desikachar, 1975).

The total soluble sugars in the grains of the 12 millet varieties ranged from 0·42% to 0·69%. This is comparable with the results obtained by Subramanian *et al.* (1981) for pearl millet.

The contents of tannin and total phenolics in Italian millet samples are low and ranged from 0·05% to 0·10% and 0·07% to 0·15%, respectively. Tannins and phenolic constituents (polyphenols) of millet have been reported to adversely affect the availability of protein in the diet (Ramachandra *et al.*, 1977). It may be concluded that such low levels of polyphenols in Italian millet may not have any effect on the utilisation of the millet protein. The tannin content of Italian millet is in accordance with the values obtained for proso millet (*Panicum milliaceum*) by Lorenz (1983).

IVPD of the whole seed flour with pepsin and papain was quite high, i.e. 95·5% in the case of pepsin and 92·9% with papain (Table 3). Similar results on the IVPD with pepsin have been obtained for finger millet (Ramachandra *et al.*, 1977) and sorghum (Axtell *et al.*, 1981). Such high digestibility values with pepsin can be attributed to the wide specificity of peptide bond cleavage by pepsin. The pH of incubation might also have changed the conformation of the protein substrate, thus increasing the digestibility. This effect of pH on digestibility is further confirmed in another experiment where acid treatment before trypsin hydrolysis increased the digestibility considerably.

The IVPD of whole seed Italian millet flour with trypsin is rather low with a mean value of 26·8% and may reflect the deficiency of lysine and the abundance of proline in the protein (Monteiro *et al.*, 1982). Low tryptic hydrolysis of Italian millet protein may also be due to the presence of trypsin inhibitor activity in the flour. Chandrasekhar *et al.* (1982) have demonstrated antitryptic activity in the Italian millet flour extracts. They also showed that pepsin treatment inactivated the proteinase inhibitors, which would explain the high IVPD with pepsin.

When the flour was first treated with dilute acid (pH 2·0) before trypsin digestion, the IVPD increased considerably (Table 4). This can be due to the inactivation of trypsin inhibitor by acid. Further, the susceptibility to

TABLE 3
In Vitro Protein Digestibility of Italian Millet Varieties

Variety	*Digestibility* (%)		
	Pepsin	*Papain*	*Trypsin*
CO-3	95·2	93·3	21·6
I.Se-201	95·7	89·7	23·5
I.Se-358	96·3	93·8	31·8
I.Se-480	90·5	94·9	36·9
I.Se-703	96·1	94·0	22·7
I.Se-709	96·0	91·2	23·0
JNSE-26	95·5	92·0	26·3
JNSE-53	95·9	89·7	23·5
K-221-1	96·9	93·1	23·6
KHS-1	95·8	95·6	34·9
SI-76/4	96·1	94·5	29·5
SI-80/2	95·6	92·7	24·8
Mean	95·5	92·9	26·8
SD	1·62	1·91	5·17
Range	90·5–96·9	89·7–95·6	21·6–36·9

proteolytic digestion depends on the availability of amino acid residues compatible with the specificity of enzymes. Therefore, any change in the tertiary structure of the protein brought about by denaturating agents, such as acid, which will expose enzyme-susceptible bonds will result in increased protein hydrolysis (Grau & Carroll, 1958).

TABLE 4
Effect of Preliminary Acid Treatment on IVPD of Italian Millet with Trypsin

Variety	*Digestibility* (%)	
	No treatment	*Acid treatment*[a]
CO-3	21·6	82·4
I.Se-480	36·9	90·6
SI-80/2	24·8	86·2
Mean	27·8	86·4
SD	8·07	4·10
Range	21·6–36·9	82·4–90·6

[a] IVPD with trypsin after a preliminary acid treatment of the flour was done by suspending 200 mg of flour in 25 ml of 0·075N HCl for 2 h. The pH of the suspension was adjusted to 7·6 with 2N NaOH and 2·5 ml of a solution of 0·2% trypsin was added to it and IVPD determined as described in the text.

CONCLUSION

The proximate composition of Italian millet is not very different from that of other minor millets and cereals except for calcium content. The antinutritional factors, phenolics and tannins, are present in very low amounts and presumably do not interfere with the digestibility of protein of the grain as has been shown for low tannin sorghums (Fuller *et al.*, 1966), as well as for low tannin finger millet varieties (Ramachandra *et al.*, 1977).

From the results obtained, it can be concluded that the proteins of Italian millet are easily digestible by pepsin but not by trypsin. However, pepsin acts on proteins for a very short length of time in the alimentary tract, its action coming to a stop when the contents of the stomach reach the intestine where the pH is unfavourable for the action of pepsin. Therefore, the nutritional significance of the high pepsin digestibility of the protein *in vitro* is uncertain and the proteins of Italian millet may not be satisfactory when used as the sole source of protein.

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