phyt-2-ene have been found only in milk fat. Nagy et al. (1969) reported three unidentified  $C_{20}H_{40}$  hydrocarbons in bovine liver. It is interesting that they found the fully saturated phytane as the major component of the C-20 hydrocarbon fraction. Kuksis (1964) found unidentified unsaturated hydrocarbons in the C-19 to C-20 region of his chromatograms of the hydrocarbons of seed oils. It therefore seems likely that unsaturated phytane derivatives are much more widely distributed in nature than has hitherto been suspected. Their function is still obscure.

### ACKNOWLEDGMENT

We thank Olaf Onder for carrying out the hydrogenations and the Department of Organic Chemistry, Melbourne University, for the use of their facilities. We also thank Graham Thornton for help in obtaining the mass spectra and E. H. Ramshaw for helpful comments on the manuscript.

## LITERATURE CITED

Ackman, R. G., J. Chromatogr. Sci. 10, 243 (1972). Aplin, R. T., Coles, L., Chem. Commun., 858 (1967).

- Baxter, J. H., Milne, G. W. A., Biochim. Biophys. Acta 176, 265 (1969).
- Bierl, B. A. Beroza, M., Aldridge, M. H., J. Chromatogr. Sci. 10, 712 (1972). Blumer, M., Thomas, D. W., Science 147, 1148 (1965). Clarke, J. R. P., Fredricks, K. M., J. Gas Chromatogr. 5, 99
- (1967)
- Flanagan, V. P., Ferretti, A., J. Lipid Res. 14, 306 (1973)
- Forss, D. A., Stark, W., Urbach, G., J. Dairy Res. 34, 131 (1967). Forss, D. A., Urbach, G., Stark, W., Int. Dairy Congr., Proc. 17th, Munich 3, 211 (1966).
- Ikan, R., Baedecker, M. J., Kaplan, I. R., Nature (London) 244, 154 (1973).
- Kuksis, A., Biochemistry 3, 1086 (1964).
- Murray, K. E., Aust. J. Chem. 12, 657 (1959). Nagy, B., Modzeleski, V. E., Scott, W. M., Biochem. J. 114, 645 (1969)
- Ristow, R., Werner, H., Fette, Seifen, Anstrichm. 70, 273 (1968). Rowland, R. L., J. Amer. Chem. Soc. 79, 5007 (1957). Sen Gupta, A. K., Peters, H., Fette, Seifen, Anstrichm. 68, 349 (1966)

- (1960). Stark, W., Smith, J. F., Forss, D. A., *J. Dairy Res.* 34, 123 (1967). Stark, W., Urbach, G., *Chem. Ind. (London)*, 413 (1974). Stark, W., Urbach, G., Hamilton, J. S., Forss, D. A., *J. Dairy*
- Res. 40, 39 (1973). Winstein, S., Lucas, H. J., J. Amer. Chem. Soc. 60, 836 (1938).

Received for review August 23, 1974. Accepted October 19, 1974.

# **Comparative Nutritive Value and Amino Acid Content of Different Extractions of** Wheat

Krishan C. Sikka, Rajinder P. Johari, Surinder K. Duggal,\* Ved P. Ahuja, and A. Austin<sup>1</sup>

The mill fractions obtained by standard milling in a Bühler automatic mill from a single wheat sample, *i.e.* whole wheat flour, bran, white flour (maida), and resultant atta (fraction left over after removing bran and white flour from whole wheat sample), have been analyzed for their nutritive value, *i.e.*, PER (protein efficiency ratio), NPR (net protein retention), and amino acid analysis. The protein quality index based on PER

In recent years a number of high yielding varieties of cereals such as wheat, rice, and maize have brought a green revolution in the Asian countries, particularly in India where the production has increased by 100% (1960 as basis). It has been reported that the bulk of the Indian population derive 72% of their dietary protein from cereals (Indian Council of Medical Research Special Report, 1953). Evaluation of the protein quality of dwarf rice varieties IR-8 and TN-1 (Taichung Native) and maize using albino rats has been reported by Bressani et al. (1971) and Mitra and Das (1971) and Mertz et al. (1965) in rice and maize, respectively. Recently Miladi and Hegsted (1972) discovered the RNV (relative nutritive value) and amino acid content of some of the milled fractions of wheat, but no biological test data based on PER, NPR, and amino acid content have been reported so far in different milled fractions used for different baking purposes. The present paper describes results of such work on amber colored dwarf mutant variety Sharbati Sonora developed at the

and NPR at a 10% protein level was found to be highest in resultant atta, followed by bran, whole wheat flour, and white flour. A chemical score based on the essential amino acid content of egg protein and an FAO provisional pattern of milk protein indicates the limiting amino acids in different fractions. The EAAI (essential amino acid index) was also calculated.

Indian Agricultural Research Institute, New Delhi (Varughese and Swaminathan, 1966).

# MATERIALS AND METHODS

Wheat Samples. Seeds of Sharbati Sonora were collected from a field experiment conducted by the Division of Genetics of the Indian Agricultural Research Institute, New Delhi. In this experiment, nitrogen at the rate of 80 kg/ha was applied to the high nitrogen responsive dwarf variety. P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied at the rate of 40 kg/ ha. For chemical analysis samples of whole meal, white flour (60-65%), bran (10-15%), and resultant atta (25-35%) were obtained by grinding wheat in a Bühler automatic mill.

Methods. Samples were dried in a hot air oven at 105° for 6 hr for the determination of moisture. The protein content of the samples was calculated by multiplying the Kjeldahl N by 5.7. The amino acid composition was studied using a Technicon automatic amino acid analyzer. Defatted samples containing 5 mg of protein were hydrolyzed by refluxing with 6 N HCl for 22 hr. After removal of acid by evaporation under reduced pressure, the residue was dissolved in 2 ml of citrate buffer (pH 2.875). An aliquot (0.4 ml) was used for the determination of amino acids according to the method of Moore and Stein (1954). Tryp-

Division of Agricultural Biochemistry, Indian Agricul-tural Research Institute, New Delhi-110012, India.

<sup>&</sup>lt;sup>1</sup>Present address: Cummings Laboratory, Indian Agricul-tural Research Institute, New Delhi-110012, India.

Protein source	g/28 days per	Protein intake, g/28 days per animal	Mean wt gain, g/28 days per animal	PER
Whole atta, 100%	193	$19 \pm 3.9^{a}$	$19 \pm 3.98^{a}$	$1.0 \pm 0.212^{a}$
Bran, 10– 15%	256	$26~\pm~7.0$	$31\ \pm\ 14.16$	$1.2 \pm 0.35$
Resultant atta, 25– 35%	230	23 ± 5.5	$34~\pm~14.00$	$1.45 \pm 0.466$
White flour, $60-65\%$	151	$15 \pm 2.8$	$10~\pm~1.5$	$0.64 \pm 0.088$
Casein	<b>2</b> 58	$26~\pm~7.0$	$66~\pm~61.2$	$2.53~\pm~1.4$

Table I. Mean Values of Food Intake, Protein Intake, Gain in Weight, and PER of Wheat Fractions and Casein at 10% Protein Level

<sup>a</sup> Standard error. Average value of one animal.

tophan was determined by the method of Spies and Chambers (1949).

**Diets.** The diets for all the biological experiments were prepared at a 10% protein level. The composition of 100 g of diet was as follows: wheat flour, calculated weight to give 10% protein; hydrogenated fat, 10 g (containing 1 mg or 100 I.U. of vitamin E); 4% mineral mixture (U.S.P. XVII 4); grind in mortar pestle 139.3 g of NaCl with 0.79 g of KI; similarly grind together 389.0 g of KH<sub>2</sub>PO<sub>4</sub>, 57.3 g of MgSO<sub>4</sub>·7H<sub>2</sub>O, 381.4 g of CaCO<sub>3</sub>, 27.0 g of FeSO<sub>4</sub>·7H<sub>2</sub>O, 4.01 g of MnSO<sub>4</sub>·H<sub>2</sub>O, 0.548 g of ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.477 g of CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.023 g of CoCl<sub>2</sub>·6H<sub>2</sub>O, adding finally the NaCl-KI mixture; 5 g of glucose and 5 ml of a complete vitamin mixture (Manna and Hauge, 1953); and 2 drops of adoxline containing vitamin A (12,000 I.U.) and vitamin D<sub>2</sub> (I.P. 2000 I.U./g was fed orally twice a week).

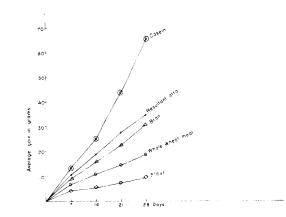
Protein Efficiency Ratio (PER). PER was determined by the method of Osborne *et al.* (1919). Weanling albino rats about 22 days old and weighing 30-40 g were divided into six groups. All groups within each experiment had the same average initial weight. Each group consisted of three males and three females.

The rats were placed in individual all wire cages with a raised platform. Water was available to them at all times. Food intake was measured every day; spilled food was collected daily and used to correct the amount of food intake. The animals were weighed twice a week for 4 weeks or 28 days.

Net Protein Retention (NPR). NPR was determined by the method of Bender and Doell (1957). One-month-old albino rats, three males and three females in each group (six groups in all), having the same initial weight were used. Wheat flour fractions in the diet were at the 10% protein level. A nonprotein diet was prepared by replacing grain flour with protein-free starch in the diet. Four groups were fed with four fractions of flour, one group was given a nonproteinous diet, and one group was given a standard casein diet. The experiment was continued for 9 days. The weight of each rat was recorded every third day. NPR was calculated as follows

$$NPR = \frac{\text{wt gain of TPG} + \text{wt loss of NPG}}{\text{wt of protein consumed}}$$

where TPG = test protein group (fractions of wheat) and NPG = nonprotein group.



**Figure 1.** Growth curves showing average weekly gains of six rats fed whole wheat meal (O), bran  $(\nabla)$ , resultant atta  $(\bullet)$ , flour  $(\Box)$ , and casein  $(\odot)$ .

Table II. Net Protein Retention (NPR) of Different Extractions of Wheat and Casein at 10% Protein Level

Protein source	Av protein intake in 10 days, g	Av wt gain, g	Av wt loss in nonprotein diet, g	NPR
Whole wheat flour, 100%	6.30	6.60	5.75	1.96
Bran, 10-15%	6.70	8.75	5.75	2.16
Resultant atta, 25-35%	7.10	11.25	5.75	2.37
White flour, 60-65%	5.70	3.75	5.75	1.66
Casein	7:20	18.70	5.75	3.50

#### RESULTS AND DISCUSSION

Table I shows food intake, protein intake, and PER. The PER values reveal that resultant atta had better protein quality than bran, whole meal flour, and white flour (Figure 1). The higher PER values of resultant atta reflect the superior protein quality of these two fractions over whole meal and white flour. Similar higher PER and higher relative nutritive values in germ and bran fractions have been reported by Miladi and Hegsted (1972) in milled fractions. The results further confirm the PER values obtained by Mitra and Das (1971) for whole wheat flour made from Sharbati Sonora.

Net Protein Retention. The NPR values of four extractions of Sharbati Sonora are presented in Table II. The values listed in Table II for test samples are highest for resultant atta, followed by bran, whole wheat, and white flour. The NPR values appear to magnify the differences in protein quality of different fractions to a greater extent than other biological determinations (PER) because the PER measures protein efficiency based on growth only, whereas the NPR measures protein efficiency based on both growth and maintenance. However, the relative order remains the same as in PER determination. Since determination of NPR is less time consuming (9-10 days) as compared to PER (28 days) and involves less laboratory work as compared to BV (biological value), it is a useful test for evaluation of protein quality. According to Bender and Doell (1957), NPR is far more accurate as a measure of protein value than PER and gives highly significant correlation with NPU (net protein utilization).

# Table III. Amino Acid Composition of Different Extractions of Wheat (g/16 g of N)

	Whole		White	Resultant
	wheat,	Bran,	flour,	atta,
Amino acid	100%	10-15%	60-65%	25 - 35%
Aspartic acid	5.18	7.61	4,13	5.24
Threonine	2.72	3.05	2.59	2.66
Serine	4.40	4.54	4.64	5.02
Glutamic acid	32.45	21.28	36.54	26.53
Proline	9.68	6.65	12.26	9.65
Glycine	3.72	5.96	3.83	3.47
Alanine	3.35	4.70	3.80	3.49
Valine	5.10	5.77	5.11	4.53
Cystine	1.76	0.92	0.86	1.13
Methionine	1.88	1.14	1.23	1.90
Isoleucine	3.40	3.62	3.93	3.30
Leucine	6.08	5.20	7.64	5.20
Tyrosine	3.12	2.18	2.86	2.35
Lysine	2.68	3.95	1.65	3.30
Histidine	2.60	3.28	2.24	3.31
Arginine	4.52	7.27	3.79	6.41
Tryptophan	1.05	0.92	0.84	0.73
Protein	14.24	16.36	13.39	13.54
(g/100 g of sample)				

sample)

Amino Acid and Chemical Score. The amino acid composition of four fractions of wheat is presented in Table III. It is observed from Table III that resultant atta is deficient to a much lesser extent in a number of amino acids as compared to the FAO pattern. The limiting amino acids in resultant atta and bran are methionine and isoleucine, whereas in whole meal and white flour lysine, threonine, and methionine are limiting. This agrees very well with the results reported by Miladi and Hegsted (1972) in bran, wheat flour, and white flour (Eggum, 1970).

A close correlation was observed between PER and the amino acid composition. Low PER obtained in the case of white flour and whole wheat flour is due to the deficiency of threonine, lysine, and methionine.

E:N, E:P, and E:T ratios, chemical scores, and EAAI in different fractions are presented in Table IV. The results show that in E:N, E:P, and E:T ratios in bran are better than in any other fraction, followed closely by resultant atta.

The results obtained for chemical score as well as EAAI (essential amino acid index) in the case of whole wheat meal agree very closely with those of Sharbati Sonora reported by Eggum (1970). As observed from Table IV PER is well correlated with chemical score in the case of white

Table IV. E:N, E:P, and E:T Ratios, Chemical Score,
and EAAI in Different Extractions of Wheat

	mg/g		Chemical EAAI, <sup>a</sup> score,		Chemical score,	
Feedstuff	E:N	E:P	E:T	%	%	,
Whole meal, 100%	552	349	355	65.64	42.50	63.8
Bran, 10– 15%	680	374	404	63.84	37.00	51.8
White flour, $60-65\%$	513	351	339	57.87	26.20	39.3
Resultant atta, 25-35%	581	347	367	63,55	37.20	52.0
Egg FAO provi- sional pattern milk				100.0	100.0	100.00

 $^{a}$  EAAI (essential amino acid index) is based upon the ratios of the amounts of essential amino acids in a protein relative to their amounts in whole egg protein (Oser, 1951). Chemical score is the percentage of the most deficient essential amino acid in the protein as compared to the requirement pattern (Mitchell and Block, 1946).

flour, bran, and resultant atta but this is not true in the case of whole wheat meal.

## ACKNOWLEDGMENT

The authors are grateful to M. S. Naik and H. K. Jain of the Indian Agricultural Research Institute, New Delhi, for keen interest and encouragement and for providing facilities.

#### LITERATURE CITED

- Bender, A. E., Doell, B. H., Brit. J. Nutr. 11, 140 (1957). Bressani, R., Elias, L. G., Juliano, B. O., J. Agr. Food. Chem. 19, 1028 (1971)
- Eggum, B. O., International Atomic Energy Agency, IAEA-SM-132/40, 1970, p 289.
- Indian Council of Medical Research (ICMR), Special Report, No. 25, 1953, p 25.
- Manna, L., Hauge, S. M., *J. Biol. Chem.* **202**, 91 (1953). Mertz, E. T., Vernon, O. A., Bates, L. S., Nelson, O. E., Science 148, 1741 (1965).

- 148, 1741 (1965).
  Miladi, S., Hegsted, D. M., Cereal. Chem. 49, 119 (1972).
  Mitchell, H. H., Block, R. J., J. Biol. Chem. 163, 599 (1946).
  Mitra, G. N., Das, N. B., J. Agr. Food. Chem. 19, 927 (1971).
  Moore, S., Stein, W. H., J. Biol. Chem. 211, 892 (1954).
  Osborne, T. B., Mendel, L. B., Ferry, E. L., J. Biol. Chem. 37, 223 (1919).

- Oser, B. L., J. Amer. Diet. Ass. 27, 396 (1951). Spies, J. R., Chambers, D. C., Anal. Chem. 21, 1240 (1949). Varughese, G., Swaminathan, M. S., Curr. Sci. 35, 469 (1966).
- Received for review January 26, 1973. Accepted September 5, 1974.