

The fate of ethephon in the bovine is speculative. It is possible that the compound may decompose in the presence of rumen fluid, with the release of ethylene gas, dihydrogen phosphite, and chloride ions. This reaction is thought to occur in plant tissues (Technical Service Data Sheet, 1969). The rapid degradation of ethephon in rumen fluid may explain the presence of only 9.8% of the intact compound in urine. Since ethephon is not fat-soluble, its absence in milk at low feeding levels is not surprising. Based on past analysis of field-treated fruit and pomace in this laboratory, concentrations of ethephon in harvested apple fruit and pomace would expectedly be well below 5 ppm, the dose level in this investigation. Residues of ethephon in pomace therefore would not be expected to cause milk contamination if the pomace was included in the dairy ration.

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## Free Amino Acids of Pakistani Wheat Varieties

Free amino acids content of eight Pakistani wheat varieties has been determined by paper chromatography. In all, 13 amino acids have been found in all the varieties. These varieties differ both qualitatively and quantitatively in free

amino acids. Glutamic acid, aspartic acid, phenylalanine, cystine, tyrosine, and valine are common to all varieties. Nortino variety contains the greatest number and amount of free amino acids, while C-271 and C-273 contain the least.

Amino acids, one of the most important constituents of our daily diets from a nutritional viewpoint, exist almost invariably in all the food products, both in the free as well as the bound form. Proteolysis and the partial or complete inability of any amino acid to take part in protein synthesis may be the reasons for the presence of free amino acids in foods. Free amino acids have been used as an index of maturity in certain fruits (Rockland and Underwood, 1954). In some foods, these have been taken as an index of decomposition (Fuks and Wierzchowski, 1967), while in bovine muscle their greater quantities have been associated with tenderness (Field and Chang, 1969).

Free amino acids of various food products have been reported in the literature (Coussin and Samish, 1968; Elahi and Khan, 1971; Ghadimi and Pecora, 1963; Petrovlovskii and Troyan, 1970; Tinsley and Bockian, 1959; Wolfgang *et al.*, 1970). Data concerning the free amino acid content of Russian (Ekimovsky and Somin, 1970) and American (Hoseney and Finney, 1967) wheat varieties are present in literature.

In this paper, the free amino acids of eight ripe Pakistani wheat varieties have been reported.

#### EXPERIMENTAL SECTION

**Apparatus and Reagents.** Paper chromatography was carried out on Whatman No. 1 paper, using glass chamber (21 × 8 in.). A Beckman DU spectrophotometer was used for optical density measurements.

Ninhydrin reagent was prepared by dissolving 0.5 g of commercial ninhydrin in 100 ml of acetone. Solvents used were of analytical grade.

**Preparation of Samples.** Pure samples of eight wheat

varieties (*i.e.*, C-271, C-273, C-591, Penjamo 62, Maxi-Pak red, Maxi-Pak white, Nortino, and Dirk) were collected on June 26, 1970, of the 1970 crop, from Ayub Agriculture Research Institute, Lyallpur. Each variety was ground into whole meal flour in the laboratory test mill after being freed of dirt and foreign seeds.

**Extraction of Amino Acids.** The extraction procedure is essentially that of Pant and Tulsiani (1968). Flour (5 g) of each variety was defatted in a Soxhlet apparatus and was well stirred in 50 ml of 70% ethanol for 30 min and centrifuged. The supernatant layer was decanted and the residue was again stirred with the solvent and centrifuged. The process was repeated until the supernatant layer gave no color with ninhydrin solution. All the ethanol extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of distilled water. The solution was used for both qualitative and quantitative purposes.

**Qualitative and Quantitative Analysis.** Free amino acids were determined both qualitatively and quantitatively by spotting 0.25–0.50 ml of the extract on Whatman No. 1 paper using one-dimensional ascending paper chromatographic techniques as described earlier (Elahi and Khan, 1971).

The following solvent systems were used: (1) propanol-water (70:30 v/v); (2) phenol-*n*-propanol-water (100:20:20 v/v); and (3) ethylmethyl ketone-propionic acid-water-*tert*-butanol (75:25:30:20 v/v).

Serine and glycine, valine and methionine were not separable in solvent 1 and were separated in solvent 2. Lysine and histidine, alanine and threonine were not separable in solvents 1 and 2 and were separated by first passing solvent 1 and then after drying solvent 3 in the same direction.

**Table I. Free Amino Acids of Various Pakistani Wheat Varieties (mg % Dry Basis)**

Amino acids <sup>1</sup>	Wheat varieties							
	C-271	C-273	C-591	Nortino	Maxi-red	Maxi-white	Penjamo	Dirk
Phenylalanine	6	6	7	7	6	7	8	4
Alanine	4			6	5	6	7	6
Glycine				5				5
Leucine								
Serine		4	3	3	4			
Threonine								
Proline			8					
Lysine				2			2	
Valine	3	3	4	5	3	5	4	6
Histidine								
Tyrosine	4	4	4	4	5	5	4	5
Methionine								
Cystine	3	3	4	3	3	3	4	3
Aspartic acid	5	5	6	7	7	9	9	7
Glutamic acid	4	6	6	5	6	6	7	8
Asparagine				7			6	8
Arginine				8	7			4
Sum	29	31	42	62	46	41	51	56
Total amino acids found experimentally	27	29	43	63	47	39	50	54

Cystine and cysteine are not separable in any of the solvent systems used and are determined in terms of cystine.

**Estimation of Total Free Amino Acids.** Ninhydrin reagent (0.5 ml) was added to flour extract (0.5 ml) and diluted to 10 ml with ethanol. It was kept at 70° for 0.5 hr and the intensity of the color was measured at 570  $\mu$  using the mixture of ethanol and extract (9.5 and 0.5 ml) as the blank. The concentration of total free amino acids in terms of phenylalanine was determined from a standard curve prepared for phenylalanine under similar conditions. The estimates of both total and individual free amino acids (Table I) are averages of duplicate results.

#### RESULTS AND DISCUSSION

Table I shows the free amino acids of various wheat varieties. C-271, C-273, and Maxi-Pak white contain the minimum (*i.e.*, 7) and Nortino contains the maximum number (*i.e.*, 12) of free amino acids. In all, 13 amino acids have been identified. It will be observed that phenylalanine, valine, tyrosine, cystine, aspartic acid, and glutamic acid are common to all the varieties. Leucine, methionine, histidine, and threonine have not been identified in any variety, even on spotting more concentrated samples. Proline has been identified only in the C-591 variety.

It also includes the total and individual free amino acid contents, which were eluted from the paper and determined from a standard curve prepared for each acid under similar conditions. It will be observed that C-271 possesses the lowest (*i.e.*, 28 mg %) and Nortino possesses the highest (*i.e.*, 60 mg %) content of total free amino acids. The arithmetic sum of individual acids of different wheat varieties is almost equal to the total content as determined experimentally. Aspartic acid has been found in the greatest concentrations and lysine in the lowest concentrations among the amino acids identified. It is apparent from Table I that the wheat varieties differ both qual-

itatively and quantitatively with respect to free amino acids.

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