Amino Acids, Sugars, and Inorganic Elements in the Sweet Almond (*Prunus amygdalus*)

F. Saura Calixto,* M. Bauza, F. Martinez de Toda, and A. Argamenteria

A study of amino acids, sugars, and mineral elements is carried out with the sweet almond cultivated in Mallorca (Spain). Seventeen amino acids are determined by ion-exchange liquid–liquid chromatography. Glutamic acid is the most abundant, and the essential amino acids determined constitute 34% by weight of the total amino acids determined. The absence of reducing sugars is noted by several methods, and the content of sucrose is found to be 5.52%. The elements Fe, Ca, K, Na, Mg, Cu, Zn, Mn, P, Co, and Mo are determined by atomic absorption and colorimetric methods. Cobalt and molybdenum have not been previously cited in this fruit by other authors.

Worldwide cultivation of the almond has experienced a rapid growth in recent decades. The area devoted to this cultivation has quadrupled between 1960 and 1974, and the United States and Spain are now the principal producer countries with approximately 50 and 27% of the world's total production, respectively.

The major countries of consumption, expressed in grams per inhabitant per year, are Switzerland (700–725) and Norway (525–550). The consumption in Spain and the United States was 500 and 200 g, respectively, according to data available in 1976 (Zaballas Zayas, 1976). An annual consumption increase of 7% is now foreseen, and from 1981 demand could overcome production.

This increase in production and consumption is largely due to the excellent nutritional value of the fruit. The almonds are an important source of vegetable protein, not below 20 g of protein/100 g of almond. On the other hand, the fruit's high fat content makes it an excellent source of energy, increasing the caloric content of the diet without contributing to the formation of blood cholesterol because the main constituents are unsaturated fatty acids: oleic = 78%; linoleic = 18% (Cowan et al., 1963). Almonds are also known to be a good source of mineral bioelements.

In this paper we carried out a study of amino acids, sugars, and inorganic elements in the sweet almond from the Spanish Mediterranean island of Mallorca. Few references occur in the bibliography concerning these components. A study of oil has not been carried out because ample information on these substances is already available (Cowan et al., 1963; Nassar et al., 1977; Zuercher and Hadorn, 1976; García Olmedo et al., 1971, 1978).

Nassar et al. (1977) have identified 11 amino acids by gas chromatography, aspartic acid, leucine, and glutamic acid being named as major constituents. Results of analyses of amino acids by column chromatography and microbiological techniques on three samples of almond have been published by FAO (1970).

Valls and García Olmedo (1963) have detected sucrose in almond milk by paper chromatography. Glucose and fructose have been found by gas–liquid chromatography by Battaglini (1974). Sequeira and Leiw (1970) have identified fructose, glucose, sucrose, sorbitol, and inositol in the almond shell. Sucrose is cited as a major constituent by Zuercher and Hadorn (1976).

As for inorganic elements, the works of Klindner and Dworschak (1966), Souty et al. (1971), Romojaro et al. (1977), and Bosch Ariño et al. (1977a,b), which have determined several major elements and trace elements by flame photometry, X-ray fluorescence, atomic absorption, and colorimetric methods, should be mentioned.

EXPERIMENTAL SECTION

Studies were made with the principal almond varieties (Pons, Canaleta, and Marcona) grown on Mallorca whose surface area devoted to cultivation of the fruit represents 20% of the total in Spain. Almonds cultivated on this island are collectively known by the commercial name "Mallorca propietor".

Equipment. The determinations of amino acids were carried out with a Kontron Liquimat III automatic analyzer, ion-exchange liquid–liquid chromatograph, equipped with a Perkin-Elmer Sigma 10 integrator.

A Perkin-Elmer 703 atomic absorption spectrophotometer is used for analysis of the mineral elements except for molybdenum and phosphorus.

Quantitative analysis of sugars and determination of molybdenum and phosphorus are carried out with a Beckman Acta C III spectrophotometer.

The remaining tests are carried out with conventional laboratory apparatus.

Analytical Procedures. *General Analysis and Determination of Amino Acids.* First, the tegument of the almond nut is removed. Water is then removed by drying at a temperature of 100 °C and the oil is extracted with ethyl ether by using a Soxhlet extractor. Random samples in this form are used to determine amounts of fibers, nitrogen, and protein by the Kjeldahl method. These determinations are carried out in accordance with AOAC (1970) standards.

The hydrolysis of proteins was carried out on 50–60-mg samples without oil with 80 mL of 6 N HCl in reflux during 24 h at 110 °C. Under these conditions tryptophan is completely destroyed. Threonine and serine are partially destroyed, and it was necessary to extrapolate to zero the results of samples hydrolyzed 24, 48, and 72 h.

Analysis by liquid–liquid chromatography is carried out on the hydrolyzed samples. The elution of amino acids in the column depends on the pH of buffered solutions, the time, and the temperatures. Buffered solutions of pH 2.55, 3.05, 3.10, 4.10, and 4.80 at temperatures of 41, 25, 41, 56, and 67 °C are used. Quantitative determinations are made using to analyzer by the ninhydrin reaction. Under these experimental conditions, it is possible to separate up to 50 different compounds sensitive to ninhydrin.

Qualitative and quantitative data are calculated with retention parameters and peak areas of the chromatograms of experimental and control samples.

Sugars. Analyses of sugars are carried out on solutions of crushed almond in distilled water, which is then filtered and clarified. Some solutions are acidified to produce sucrose inversion.

Sciences Faculty, University of Palma de Mallorca, Spain (F.S.C. and M.B), and Superior Technical School of Agronomist Engineers, Polytechnical University of Madrid, Spain (F.M.d.T. and A.A.).

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Table I. General Analysis

	%
water	6.93
ash	3.05
fiber	1.91
oil	53.37
nitrogen	3.96
protein ($N \times 5.18$)	20.51

Ascending and descending chromatograms with Whatman No. 1 paper are made. Ethyl acetate, pyridine, and water (55:25:20) and 1-butanol, acetic acid, and water (4:1:5) are the solvents used. The general procedures of Smith and Feinberg (1979) and specific notes of Valls and García Olmedo (1963) are followed.

Quantitative analysis of sugars were carried out on the crude extracts by colorimetric, volumetric, and gravimetric methods. The colorimetric methods of Haas, modified by Yadey and Weisler (Dee Snell and Ettre, 1971), and Ting, described by Carballido et al. (1974), are used.

Gravimetric determinations are carried out by the Walker method (Dee Snell and Ettre, 1971), and the volumetric analyses are made by the Lane–Eynon procedure, as described by Pearson (1976). Both methods are based on the reduction of copper oxide by reducing sugars.

Mineral Elements. Samples are reduced to ash at 500 °C for 5 h, and the ashes are recovered by using a HCl–HNO_3 (5:1) mixture for all elements except molybdenum which was dissolved in a 60% solution of $HClO_4$.

Mg, Ca, Fe, Cu, Mn, Zn, and Co are determined by atomic absorption of Na and K by emission. For preparation of standard solutions and elimination of interferences, wavelengths and others experimental conditions are followed according to the specifications of Pinta (1971) for vegetable samples.

Determination of cobalt is not made directly. It is first necessary to form the complex with 8-hydroxyquinoleine according to the indications of Sandell and Onishi (1978).

Phosphorus is determined by colorimetry in the form of vanadium phosphomolybdate, the method being based on the Misson reaction (Pearson, 1976). Molybdenum is analyzed with the colorimetric method based on the formation of the complex with thiocyanate, which is extracted with butyl acetate (Dee Snell and Ettre, 1971).

RESULTS AND DISCUSSION

All results represent average values because analysis showed no significant differences among any of the three varieties mentioned under Experimental Section.

General Analysis and Determination of Amino Acids. Table I gives the characteristic data of food analysis, expressed in percentage. Reference is to almond without integument (the integument is 6.71% of the almond). These values are in agreement with data published by other authors for varieties of almond produced in other countries. The 5.18 factor for almond protein is recommended by FAO as well as several authors referred to in the literature (Carballido et al., 1969).

Table II lists the results of analysis of amino acids expressed in milligrams per 100 g of almond. Essential amino acids comprise 34% by weight of the total amino acids determined. Glutamic acid is the most abundant, and the three major nonessential constituents account for 46.4% of the total amino acids cited.

Seven chromatograms of experimental samples and two chromatograms of controls are made to obtain these values with very similar results.

Since trytophan is destroyed by acid hydrolysis, it is not included in the table. According to FAO (1970) data, referred to above, this amino acid is present in a quantity of 172 mg/100 g.

Table II. Amino Acids in the Almond

essential amino acid	mg/100 g	nonessential amino acid	mg/100 g
isoleucine	671	arginine	2095
leucine	1137	histidine	483
lysine	653	alanine	689
methionine	272	aspartic acid	1695
cystine	452	glutamic acid	4232
phenylalanine	819	glycine	938
tyrosine	713	proline	715
threonine	467	serine	578
valine	691		

total amino acids: 17 300 mg/100 g
total essential amino acids: 5875 mg/100 g

Table III. Values for Description of Almond Protein

essential amino acid	mg/g[a]	g/100 g[b]
isoleucine	114	3.27
leucine	191	5.54
lysine	111	3.18
methionine	46	1.33
cystine	77	2.20
phenylalanine	139	3.99
tyrosine	121	3.48
threonine	79	2.28
valine	118	3.37

[a] Milligrams of essential amino acid per gram of total essential amino acids. [b] Gram of essential amino acid per 100 g of protein.

Table IV. Total Sugars

method	g of sucrose/ 100 g of almond
volumetric	5.58
colorimetric	5.53
gravimetric	5.47
	av: 5.52

The ratio E/T (grams of essential amino acids per gram of nitrogen) is 1.48. Table III includes the values of the ratio A/E (milligrams of essential amino acid per gram of total essential amino acids) and the ratio of grams of essential amino acid/100 g of protein, proposed by FAO/OMS (1966). Tryptophan content is excluded in both calculations.

The sulfur amino acids cystine and methionine are the limiting amino acids in the oleaginous seeds. In the almond, methionine content is also low, but cystine content is not limiting; it is greater than that corresponding to the protein proposed by FAO and superior that in foods of animal origin, such as cows milk or human milk.

Sugars. The ascending and descending chromatograms made with different solvents show only the presence of sucrose on the neutralized samples, while the acidified samples show three spots corresponding to glucose, fructose (produced by the inversion of sucrose), and sucrose.

Quantitative analyses of reducing sugars are negative, which confirms the presence of sucrose as the only constituent.

Determination of total sugars was carried out with six samples, and the results obtained with different methods are very much in agreement as is shown in Table IV.

It can be concluded, therefore, that the quantity of sucrose is low and reducing sugars are not present.

Mineral Elements. The composition of mineral elements are listed in Table V, expressed in milligram of element per 100 g of almond. The results are average

Table V. Mineral Elements

element	mg/100 g of almond
potassium	766
phosphorus	364
magnesium	227
calcium	185
sodium	12.2
zinc	3.8
iron	2.6
copper	1.2
manganese	1.3
cobalt	4.8×10^{-3}
molybdenum	5.7×10^{-3}

values of analysis made on six samples.

We do not find bibliographic references concerning cobalt and molybdenum in the almond, and we suppose that these elements are hereby detected for the first time in this fruit. As is known, molybdenum is the essential element found is smallest proportions in vegetable matter. Cobalt, a component of vitamin B_{12}, is not an essential element and its presence may be attributed to associated microorganisms (Gomez Campo and Mellado, 1975).

As indicated in Table I, almonds have an ash content of 3050 mg/100 g. Potassium, phosphorus, magnesium, and calcium account for 1542 mg/100 g which is 50.56% of the ashes. The other essential elements Fe, Mn, Mo, Cu, and Zn account for 8.9 mg/100 g.

The high quantity of K, P, Mg, and Ca, together with the small proportion of Na and the content of the essential elements Fe, Mn, Cu, and Zn, allows us to consider the almond an excellent source of bioelements. According to Souty et al. (1971), calcium is only partially assimilable because it is precipited partly as an oxalate in the vacuoles of the fruit.

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The Nature of Freeze-Induced White Spots on Orange Segment Walls

Raymond D. Bennett* and Roger F. Albach

Exposure of oranges to freezing conditions causes formation of white spots on the walls of the fruit segments. The spots are actually located in the tissue comprising the separation zone between segments; when two adjacent segments are pulled apart, each white spot is split in half. The chemical nature of the white spot material was previously in dispute, but it has now been shown to be microcrystalline hesperidin coating the walls of cells in the separation zone. Freezing causes damage to cell membranes, and a soluble form of hesperidin located in the cell vacuoles is thereby released and crystallizes.

One of the characteristic symptoms of freeze damage in oranges is the appearance of small white spots on the segment walls of the fruit. These spots have been considered for many years to be crystals of hesperidin, a citrus flavanone glycoside. Recently Albach et al. (1977) pointed out that the previous literature on the subject (Webber, 1896; Milliken et al., 1919; Hall, 1925) provided no convincing evidence that the spots were in fact crystalline or that they were composed of hesperidin. On the contrary, Albach et al. (1977) concluded on the basis of low-magnification microscopic observations that the white spots

Fruit and Vegetable Chemistry Laboratory, U.S. Department of Agriculture, Pasadena, California 91106 (R.D.B.), and Food Crops Utilization Research Laboratory, U.S. Department of Agriculture, Weslaco, Texas 78596 (R.F.A).