BIOCHEMICAL CHANGES AT VARIOUS STAGES OF SEED DEVELOPMENT IN CHICK PEAS (*Cicer arietinum* L.)

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COMPOSITION OF CHICK PEAS

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AN ABSTRACT OF THE THESIS OF

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Title: Biochemical changes at various stages of seed development in chick peas (Cicer arietinum L.).

A study on biochemical changes during seed development of chick peas (Cicer arietinum L.) was conducted at the seed technology laboratory of the American University of Beirut, Lebanon. Seeds were collected from a field crop at 14, 21, 28, 35, and 42 days after flowering and from a sand culture crop at 14, 21, 28, and 42 days after flowering. The seed samples collected from the field were used in the analysis for proximate composition and total and free amino acids, whereas the samples collected from the sand culture were used for the mineral composition analysis.

Protein, fat, fibre, ash, and nitrogen-free-extract increased with the maturity of the seed. A steady decrease in the moisture content was observed in the seeds from the first stage at 14 days after flowering to the fifth stage of maturity at 42 days after flowering.

No definite trend in the changes of amino acid content was observed during the different stages of seed development. All the essential amino acids studied increased in quantity towards maturity except lysine. Almost all the amino acids in the seeds of chick peas and at different stages of development were found in the form of proteins. The sulfur-containing amino acids, methionine and cystine were found to be the most limiting at all stages of seed development. The nutritional value of chick peas was slightly improved as the seeds became matured.

The minerals calcium, magnesium, sodium, potassium, iron, zinc, manganese, and copper decreased in amount during seed development when reported on the basis of mg/100 g dry matter.

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I. INTRODUCTION

In the Middle East as well as in Afro-Asian and southern American countries, chick peas (Cicer arietinum L.) is grown as a grain legume and serves as a good source of cheap protein in the dietaries of many people. The average daily per caput consumption of chick peas in India and Pakistan ranges from 65 to 71 g while in South Africa, Tunisia, United Arab Republic, Mexico, and Southern Rhodesia the average daily per capita consumption of legumes in general is 13, 25, 29, 42, and 43 g, respectively (4, pp 20-29). About 15-30 percent of protein calories and eight percent of protein consumed in the world are derived from legumes in which chick peas contributes an appreciable quantity (29, pp 14-15, 4, 18). Legumes are important in the diet as a good source of thiamine, niacin, calcium, and iron, which are essential from the nutritional point of view and play an important role in carrying out certain key reactions in metabolism.

The protein content of chick peas ranges from 17-25 percent which is about double that of the cereals and slightly higher than those of meat, fish, and eggs (4, pp 37-38). However, the contribution of a dietary protein to the requirements of the human beings or animals

depends not only on the quantity of the protein present in the diet, but also on its quality; mainly, on its amino acid composition (35, 36). A diet deficient in a single essential amino acid will cause a decrease in the amount of protein synthesized in the body to the level of the deficient essential amino acid, although all other amino acids may be present in adequate amounts (30, pp 19-22). In 1957 the FAO Committee on Protein Requirements proposed a hypothetical reference protein to be used as a standard for measuring the quality of unknown protein. The quality of an unknown protein is measured by comparing the level of the essential amino acids in the protein in question against the level of the essential amino acids in the reference protein. The provisional pattern of amino acids in the reference protein reported by the FAO 1957 was found to be inadequate. Hence in 1965 the FAO/WHO Experts Group on Protein Requirements emphasized the need for a more detailed knowledge of the amino acid composition of protein and published a new recommended method for scoring the quality of an unknown protein. The values reported by this group were based on the pattern of the essential amino acids in the egg protein.

Large quantities of chick peas are consumed as a vegetable source at different stages of maturity when the seeds are still green and tender. These immature green seeds contain carotene and ascorbic acid, which are almost

absent in dry seeds.

The purpose of the present study was to determine the changes in moisture, protein, fat, fibre, ash, total and free amino acids, and mineral composition during the seed development of chick peas. In addition, protein quality evaluation on the basis of the FAO 1957 (1) and FAO/WHO 1965 (2) scoring patterns was performed.

II. REVIEW OF LITERATURE

Very few attempts have been made to investigate the distribution of constituents in the seed during various stages of development. These few investigations are mainly confined to the study of nitrogenous compounds and carbohydrates in peas. Some workers, however, have reported the results of chemical analyses in different other crops at various stages of ripening.

Changes in Carbohydrates

Carbohydrates are the chief storage forms of most seeds. Starch is the main form of carbohydrates in all the food grains and legumes. The hemicelluloses occur in the endosperm of the palms and in cotyledons of Lupinus spp., Primula spp., and Impatiens spp. In addition, various sugars such as glucose, fructose, sucrose, raffinose, and stachyose occur in greater or smaller amounts in most seeds.

Crocker and Barton (8, pp 153-155) have reported that starch and hemicellulose are the two common storage forms of carbohydrates in seeds where a high percentage of carbohydrate is present. Turner et al. (40) found that during the first ripening stage the simple sugars consist

in the main (about 90 percent) of sucrose, the remainder consisting of glucose and fructose in equal amounts. Similar results have been obtained by Danielson (10). Turner and Turner (39) worked on the developing pea seeds and found that there is a period about 21 days after flowering in which the starch content rises rapidly and sucrose content falls. Jodidi (24) reported that in general the mature peas have a large percentage of starch and a small percentage of sucrose. Turner and Turner (39) also reported that there was a linear relationship, over most of the period of development, between the rate of starch synthesis and starch phosphorylase activity. Bisson and Jones (6, pp 95-104) found in the hulls of the developing pods of peas an early accumulation of starch which later disappeared almost completely, presumably through translocation to the seeds. The hexoses and sucrose sugars in the hulls showed similar changes with time, but were present in considerably greater amounts than starch. Sucrose sugar reached an early maximum in the seeds also, and declined later in maturity to a steady value. Starch per seed increased throughout development, slowly at first but very rapidly later. Crude fibre consisting mainly of cellulose, increased steadily throughout in both hulls and seed. Evans (15) found that there was a steady increase in the amount of starch, sucrose, and ash in the corn Kernel during development. There was

also an increase in the percentage of reducing sugars from 15-22 days after silking, then a decrease to a constant value after 36 days. Sugars decreased as the seed advanced in maturity and then reached a constant value in about 43 days. Pickett (31, pp 212) observed a decrease in the percentage of sucrose and starch as the pea seed develops. The total percentage of sugars and starch approaches a constant value in the fortnight preceding maturity. The hemicellulose fraction increases throughout the development of the seed. Hoover (20) studied the effect of maturity upon protein, starch, and total and reducing sugar contents in seed of the southern peas (Vigna sinensis) when harvested at six different stages of maturity. The first harvest was made when the seeds had developed enough growth so they could be separated from the pericarp. Subsequent harvests were made at two-day intervals thereafter until the sixth or the final stage was harvested. An increase in the starch contents and decrease in the total and reducing sugar contents with the advancement of maturity of southern peas was observed. Starch increased with maturity from 29.2 percent in the first maturity stage to 48.0 percent in the fifth. Total sugars decreased from 10.38 percent in the first stage to a low level of 5.20 percent in the fourth. A rapid increase in total sugars was observed between the first and second stages, representing a 36 percent decrease within a two-day

period. McKee et al. (27, pp 146-149) found that developing pea seeds gained more carbohydrate than the amount lost by hulls. The amount of starch synthesized was found to be 14 mg per gram of fresh weight and 81 mg per gram of dry weight after 14 days of tagging while it was 28 mg and 142 mg per gram of fresh and dry weight respectively after 23 days of tagging. The increase in starch content in the seed was followed by a decrease in soluble carbohydrate contents, after which the seeds ceased to accumulate water. Danielson (9) determined sugar and starch contents at different stages of ripening of pea seeds and found that sugar is accumulated in the pea seed during ripening and at the end of the ripening period the sugar concentration decreases and starch is synthesized. Crocker and Barton (8, pp 153-155) reviewed the work of Koblet, who has studied the chemical changes taking place in the developing wheat grain and has reported that the amount of starch per 1000 grains increases rapidly up to the 35th day or the milk stage, thereafter practically no increase was observed. Soluble sugars are reported to fall rapidly at first and then slowly to reach a minimum per 1000 grains at about the milk ripe stage. Danielson (11) analysed the whole pea seed, embryo, and integuments and found a linear relationship between sugar and water content in the embryo at a stage before the commencement of natural dehydration of the seeds. Verma et al. (42) have reported

an increase in the carbohydrate content of the Bengal gram seed during ripening both on fresh and dry weight basis.

Changes in the Nitrogenous Substances

In a matter of a few weeks, ripening seeds synthesized large amounts of proteins from soluble nitrogenous substances drawn from other parts of the plant. Two types of proteins are found in the seeds, the metabolically active proteins which include enzyme proteins and nucleo proteins and the metabolically inactive ones which include the storage proteins. The storage or the reserve proteins constitute the major portion of the proteins in seeds. For most seeds, except cereals, these proteins are globulins. The cereals have two major proteins in their storage tissues, the prolamins and the glutelins. Cereal embryos also have globulins but since the embryo is a small portion of the seed, the globulins constitute a small proportion of the cereal proteins.

McKee (26, pp 589-590) reviewed the work of the earliest worker Emmerling, who has reported that non-protein nitrogenous substances are translocated from hulls and other plant parts to the seed of broad bean (Vicia faba) during seed development. The increase of protein nitrogen in the seed was accompanied by a decrease of non-protein nitrogenous substances. Similarly McKee (26, pp 591-592) reviewed the work of Pfenninger with Phaseolus

vulgaris, who reported a great increase in the total nitrogen per seed and a corresponding decrease in the total nitrogen per hull. The uptake per seed, however, was approximately twice the loss per hull, so it was concluded that the great bulk of the nitrogen of the seed came from other parts of the plant. Bisson and Jones (6, pp 95-104) recorded at various stages of development the amount of different constituents in the hull and in the total number ofseeds it contained. It was found that the contents of nitrogen, sugar, starch, and ash in the hulls showed an early maximum, and declined later, presumably as a result of translocation to the seeds. The increase in both starch and nitrogen in the seeds of a pod was between four and five times the loss by the hulls, demonstrating a considerable transfer of these materials from other parts of the plants to the developing seed. However, the exact origin of the material translocated to the seed in these experiments was not determined. Zeleny (43) showed that zein is nearly absent in the early milk stage of the corn kernel, but is synthesized at a rapid rate as the grain approaches maturity. It was also found that the rapid increase in the ratio of zein nitrogen to total nitrogen is almost exactly paralleled by the decrease in water soluble non-protein nitrogen. Frey (17) reported that as the percentage of total protein of the corn increased, the zein was increased, but tryptophan and valine

decreased with an increase of the total protein. Danielson (12) found that mature pea seeds contain besides albumin the two distinct globulins legumin, and vicilin. Danielson and Lis (14) determined tyrosine, tryptophan, arginine, histidine, lysine, aspartic acid, and glutamic acid in vicilin, legumin, and albumin of mature pea seeds. It was reported that vicilin and legumin have different amino acid composition and are well defined as different globulins. The amino acid composition of the albumin was also quite different from that of the globulins. Danielson (13) while studying the synthesis of different types of proteins in developing pea seeds, found that globulins and albumins were synthesized independently of each other, and that the globulins, vicilin, and legumin were synthesized at different rates, the relative content of vicilin decreased as ripening proceeded. It was also noted that protein synthesis occurred in the detached unripe seeds at room temperature, but ceased when the seeds were preserved by cooling at -20°C. Further it was reported that absolute amounts of all nitrogenous substances increase during the ripening process of pea seed, and that high molecular nitrogenous substances were found at an early stage of seed development and the amount of albumin increases slowly at a constant rate. Raacke (32) analysed pea seeds at seven different stages of development according to weight, and reported that in the early

stages the incoming nitrogen was distributed almost equally among the different fractions. At stage five there was a decrease in the amino and amide nitrogen with a concurrent increase in the peptide and protein nitrogen. At stage six the peptide nitrogen also declined and only the protein nitrogen increased. Raacke (33) worked on cotyledons and seed coats of Pisum sativum at four different stages of development and reported that as ripening progresses the nitrogenous material of the seed coat and the endosperm are absorbed by the cotyledons. It was also found that amide nitrogen is incorporated gradually but completely into the protein of the cotyledons. Raacke (34) analysed pods of Pisum sativum at seven different stages of development and reported that the protein is first formed in the pods, then broken down and transformed to the developing seeds in the form of amide and peptide. McKee et al. (27, pp 146-149) found a steady increase in the total and protein nitrogen per seed while marked fluctuations were found in the hulls, with a subsequent decrease later in the development period of peas. Twenty days after tagging the increase in protein nitrogen per seed remained approximately linear. The average daily increase from two season's data was 0.32 mg protein nitrogen. The maximum content of soluble nitrogen per seed was reached at 22 days after flowering. McKee et al. (28) collected seven samples at two to three day intervals over a period of 11 to

27 days after flowering in the first growing season and nine samples at three to four day intervals over a period of 14 to 40 days after flowering in the second growing season. The following soluble nitrogenous constituents, alpha alanine, gamma amino butyric acid, arginine, aspartic acid, glutamic acid, glycine, histidine, homoserine, leucine, methionine, methionine sulphone, methionine sulphoxide, serine, threonine, trigonelline, tyrosine, urea, and valine were found both in hulls and seeds of all the samples. In seeds, pipecolinic acid disappeared at the 27th day after flowering in the first season and at the 26th day in the second season. Proline was detectable only up to 17 days in the first season and 14 days in the second season. In the second season B-alanine disappeared after the 29th day and glutamine was not detectable in the samples from the 26th, 29th, and 32nd days old seed. Cysteic acid was undetectable in the first season but was detectable up to the 35th day in the second season. Asparagine was detectable in all seed samples of the first season but was undetectable in the samples from 32 and 35 days in the second season. All the amino acids decreased in concentration over the period of 23 to 32 days. Bressani and Conde (7) studied changes in the chemical composition and the distribution of nitrogen of maize at nine different stages of development. The first sample was harvested ten days after flowering and subsequent samples were taken at weekly intervals.

Amino acids, arginine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, and tryptophan were found in all the samples collected from 10 to 65 days after flowering. It was reported that lysine decreased from 0.32 to 0.23 g per gram of nitrogen from the 10th to the 65th day. Methionine also was found to decrease. Isoleucine increased from 0.16 to 0.32 g per gram of nitrogen from the first to the last sample collected. Leucine increased almost four fold from the 10th to the 65th day after flowering. The phenylalanine and arginine doubled in concentration during the maturation of the corn. Tyrosine, on the other hand, after a small initial increase remained constant throughout the ripening period. The tryptophan content of the corn kernel increased slightly from the 10th to the 16th day, decreasing again to constant values beyond the 23rd day after flowering. Holmes (29) determined amino acids, arginine, histidine, lysine, threonine, valine, leucine, isoleucine, phenylalanine, tryptophan, methionine, tyrosine, alanine, aspartic acid, glutamic acid, and glycine by paper chromatography, on the whole protein preparation of the mature seeds of pea. It was reported that arginine was the chief amino acid liberated on the hydrolysis of the protein from pea seeds. Hyde (22) found the same amino acids and amides studied by Holmes (29) in the mature pods and seeds of pea. It was reported that glutamine, alpha alanine, gamma amino butyric

acid, and threonine predominated, while glycine, pipe colinic acid, cystine, methionine, phenylalanine, and tyrosine were present only in small quantities. The main difference in the distribution was the predominance of asparagine in the hulls and arginine in the seeds of mature peas. Auclair and Maltais (3) reported the occurrence of gamma amino butyric acid and also identified an unknown spot as B-amino isobutyric acid, in addition to the most of the amino acids, reported above by Hyde (22) and Holmes (29). Bisset (5) recorded homoserine, citrulline, and alpha amino butyric acid in addition to the above mentioned amino acids in the ripening pea seeds 20 days after flowering.

Changes in Phosphates and Other Constituents

Phosphates play an important role in different reactions in seeds. Phosphate is required for the formation of nucleic acids, which are connected with protein synthesis and the genetic make up of the cell. The various phosphate sugars and nucleotides are important in the energy-producing-processes during seed germination. Phytin, the calcium and magnesium salt of inositol hexaphosphoric acid, may constitute about 80 percent of the total phosphorus in seeds and the remaining 20 percent are found as nucleic acids, phospholipids, phosphate esters of sugars and nucleotides and inorganic orthophosphate. Many workers

such as Fowler (16), McKee et al. (27), Rowan and Turner (37), and Verma and Lal (41) have studied changes in the phytin contents in relation to the other organic and inorganic phosphates in the maturing peas. Fowler (16) studied changes in the inorganic orthophosphate in the maturing peas and reported that the changes appeared to take place in three stages. In the first stage 28 days after blossom, the orthophosphate content was initially high and fell rapidly, while the phytin content rose. In the second stage 35 days after blossom, the orthophosphate content rose and the phytin fell sharply but began to rise again at once. In the third and the final stage 42 days after blossom, the orthophosphate fell and the phytin slowly rose. McKee et al. (27, pp 146-149) has also reported a decrease in the initial contents of the inorganic phosphate followed with a decrease in ester phosphate and then an increase at the later stages of seed development. Recently Verma and Lal (41) have studied the distribution of different phosphorus fractions in the seed and pod cover of Bengal gram at different stages of growth and development. A continuous increase in the amount of acid soluble, total and phytin phosphorus per 100 seed over the entire period of seed development was reported. The inorganic phosphorus remained nearly constant up to the 30th day after flowering and then declined to a value which was only about one half of that at the initial stage

of sampling. The phosphotide phosphorus fraction increased slightly up to the 35th day, after which it remained nearly constant till maturity. It was further reported that the acid soluble phosphorus, as a percentage of total phosphorus, declined slightly over the period of sampling; but its main components, the inorganic phosphorus and phytin phosphorus, showed very marked changes. Lal et al. (25) have studied the distribution of the nutrients in the seed parts of Bengal gram. The embryo was found to be the richest part of the whole seed while the seed coat was extremely low in most of the constituents except calcium. The cotyledons, on the other hand, being the principal component and fairly well balanced in their chemical composition account for almost the entire value of the seed. Verma et al. (42) have studied changes in the chemical composition of the seed parts on fresh weight basis during ripening of Bengal gram, and reported that total nitrogen, ether extract, ash, crude fibre, carbohydrates, calcium, iron, and phosphorus increase with maturity. On dry weight basis, however, calcium and carbohydrates are the only constituents which increase during ripening. As maturity progresses, all constituents of the seed coat decrease except calcium, iron, and fibre which increase with maturity. Evans (15) studied changes in the bio-chemical composition of corn kernel during development and reported a steady decrease in moisture

percentage and an increase in the dry matter, crude protein, starch, fibre, fat, and ash from silking to maturity. Similar results were also reported by Bressani and Conde (7).

In summary, the carbohydrates are the chief storage form of most seeds. Starch and hemicellulose are predominant in seeds where a high percentage of carbohydrates is present. The starch content increases towards maturity at the expense of the simple sugars which are relatively high at the early stages of seed development. Large amounts of proteins are synthesized in the seed from soluble nitrogenous substances drawn from other parts of the plant. In legume seeds such as broad-beans, peas, and beans the hulls of the fruit act as a temporary storage place for the non-protein nitrogen. Phytin constitutes about 80 percent of the total phosphorus in seeds. It increases towards maturity mainly at the expense of inorganic orthophosphate. Nitrogen, ether extract, ash, crude fibre, and carbohydrates increase during ripening of seeds.

III. MATERIALS AND METHODS

Seeds of chick peas (*Cicer arietinum* L.), variety local, were planted in the field at the Agricultural Research and Education Center in the Bekaa, and in sand culture in the green house at the American University of Beirut, Lebanon during 1967-68. Four harvests at different stages of development were taken from the sand culture and five from the field planting.

Field Crop

Forty rows, each five meters long, spaced 50 cm apart were planted with chick peas spaced 25 cm within the row. The plants were irrigated weekly with the furrow irrigation method. The soil was fertilized with 12 kg P_2O_5 and 12 kg N before planting. An additional 4 kg of N were added one month after planting. Many plants were killed by the fusarium root rot disease that appeared after flowering time. Open flowers were tagged at the peak of flowering time. More than 2,000 flowers were tagged in one week. The first seed harvest was made two weeks after tagging and the subsequent five harvests were made at one week intervals. Immediately after harvesting the seeds were separated from their pods, weighed, and stored below $0^{o}C$.

Sand Culture Crop

White mountain sand with a pH above eight was soaked in 30 percent concentrated hydrochloric acid, then washed in water until the pH of the sand solution was lowered to seven. One hundred and sixty round metal cans were filled with sand after having their insides lined with polyethylene bags to check any possible metal contamination. Prior to the addition of sand, three holes at the bottom of each can were made to facilitate water drainage.

Four seeds of chick peas were planted in each can, and pre-soaked seeds were used to facilitate rapid germination. One week after germination the seedlings were thinned down to two plants per can. Hoagland's (18) complete nutrient solution was applied two weeks after germination, but subsequently the plants were supplied with the nutrient solution every week up to the end of the experiment. Deionized water was supplied when required.

Flowers in chick peas continued to appear almost to the maturity of the crop. Hence, seeds even on the same branch of the plant differ considerably from each other in their physiological age. Thus, to obtain representative seed samples, open flowers were tagged. Samples were taken from the pods set on the tagged flowers only.

The first harvest was made two weeks after tagging and subsequent harvests were taken at one week intervals.

The fourth harvest was done six weeks after tagging.

Further, to eliminate any plant to plant variation, a few pods were harvested from every plant at each stage of sampling. Immediately after the pods had been picked, they were carefully brushed free of any adhering sand, and the seeds were separated from their pods, weighed, and stored below $0^{o}C$.

Chemical Determinations

Proximate composition

Moisture content, crude fat (ether extract), crude fibre, and ash were determined in duplicates according to the methods described in the Association of Official Agricultural Chemists Handbook 1965 (21). Nitrogen was determined by a modified micro-Kjeldahl method; crude protein was calculated by multiplying nitrogen percent in the sample by 6.25; nitrogen free extract was calculated by difference (30).

Total amino acids

Seed samples were freeze dried and ground to a fine powder in an electric mill. Duplicate samples of the fine ground material were weighed, one for the determination of N content and the other to be hydrolyzed for amino acid analysis. As described by Pellett (30, pp 5), a quantity of the sample containing about 12 mg nitrogen was accurately weighed on an analytical balance, and then transferred into

a 1,000 ml round-bottom flask. Traces left on the weighing paper were washed into the flask with distilled water from a wash-bottle. Then 300 ml of six N HCl were added, and the whole preparation was boiled for 24 hours under reflux on a GLAS-COL electric mantle heater. A very slow stream of purified nitrogen (N_2) was passed through the boiling mixture to prevent any oxidation during hydrolysis. At the end of the hydrolysis period the hydrolyzate was cooled to room temperature, and filtered under vacuum through a medium porosity sintered-glass funnel of suitable size.

The purpose of this filtration was to remove humin, the decomposition product of tryptophan, and other brown colored materials that resulted from the decomposition of carbohydrates and other components of the material being analyzed. A light brown-colored filtrate was collected, evaporated to dryness on the Craig rotary evaporator, washed twice with a few ml of distilled water, and re-evaporated to dryness. This was then dissolved in a few ml of sodium citrate buffer pH 2.2 and filtered through Whatman filter paper No. 4 to remove any further residue. The volume was ultimately made up to 25 ml and kept under N_2 gas in a deep freeze until analyzed.

Aliquots of the protein hydrolyzates, 0.5 ml each, were analyzed on an automatic amino acid analyzer, Phoenix, Model K-8000 based on the method derived by Spackman *et al*.

(38). Three types of water-jacketed columns packed with resin Dowex-50 were utilized for the separation of the amino acids. One short column (15 x 0.9 cm) was used to separate the basic amino acids (lysine, histidine, and arginine); the other two long columns (150 x 0.9 cm) were used for the acidic and neutral amino acids (cysteic acid, methionine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, isoleucine, leucine, tyrosine, and phenylalanine).

The amino acids present in the hydrolyzates were chromatographed as a series of peaks; the areas of these peaks were proportional to the quantities of the amino acids analyzed. A simplified method was used for calculating the peak areas (Phoenix Instruction Manual). This method involves counting the number of dots, recorded at fixed time intervals around the upper half of the curve and multiplied by the net height of the peak. The values so obtained were compared with those for similar peaks for a standard solution containing one micromole quantities of each amino acid, chromatographed under the same conditions. The ratio of the peak area calculated for the unknown quantity of an amino acid to that of standard was the amount in micromoles present in the aliquot analyzed. Using a dilution factor and considering the exact amount of nitrogen contained in the total volume of the hydrolyzate as well as the molecular weight of each specific amino

acid, the amount in mg/gm nitrogen was calculated as follows:

$$\frac{\frac{H \times W}{C} \times M.W. \times F}{Wt.\ N} = \text{Mg amino acid/gm N}$$

H = Net height of the peak

W = Number of dots around the upper half of the peak

M.W. = Molecular weight of the amino acid

F = Dilution factor, considering the total volume of the hydrolyzate and the aliquot analyzed

Wt. N = Total amount of nitrogen (mg) present in the hydrolyzate.

Free amino acids

A quantity of fresh sample containing about 12 mg nitrogen was accurately weighed and then transferred into a blender, containing 100 ml one percent picric acid. The mixture was blended for three minutes to precipitate all the protein, which was then removed by centrifugation. The supernatant liquid was passed through 2 x 8 Dowex-4 cm column to remove the yellow color of picric acid. The total volume collected was recorded and 50 ml of it were concentrated to dryness on the Craig rotary evaporator. This was then dissolved in a few ml of sodium citrate buffer pH 2.2 and filtered through Whatman filter paper No. 4 to remove any further residue. The volume was ultimately made up to ten ml. The separation and identification of the free amino acids were done as mentioned above.

Protein quality scores

A number of methods have been proposed which will score protein on the basis of their content of essential amino acids. The two procedures recommended by FAO 1957 (1) and FAO/WHO 1965 (2) were used in this study.

The provisional amino acid pattern of the reference protein published by the FAO 1957 Committee contained the hypothetical levels of essential amino acids (Table 1). Comparison was made between the amount of each essential amino acid in the protein of chick peas at five different stages of seed development expressed as percentage of their respective value in the reference protein. The lowest percentage obtained was assigned as the score of that protein.

The provisional pattern of amino acids in the reference protein reported by the FAO 1957 Committee was found to have limitations. Hence, in 1965 the FAO/WHO Expert Group on Protein Requirements published a new recommended method for scoring the quality of a protein. The values reported by this group were based on the pattern of the essential amino acids in the egg protein (Table 2).

Mineral composition

The method of wet digestion as described by Jackson (23) was used. One gram of the freeze dried ground samples was predigested overnight with 17 ml concentrated nitric acid in a 250 ml beaker.

The predigested material was covered with a watch glass and digested with three ml 72 percent perchloric acid on a hot plate under a hood. The mixture was brought rapidly to a temperature of 180°C to 200°C. The digestion was continued until the acid liquid was largely evaporated, and digestion was stopped when the residue in the beaker was clear white and slightly moist with acid. The digestion was completed and the residue was filtered into a 100 ml volumetric flask and made up to volume using hot distilled water. Glass redistilled water and analytical grade chemical reagents were used throughout the micro nutrient analysis to avoid any possible contamination.

Standards for each element were prepared to compare the readings. Calcium, magnesium, sodium, potassium, iron, manganese, zinc, and copper were determined in the digest using atomic absorption flame photometry - Perkin Elmer, Model 303.

Table 1. Essential amino acids in the FAO 1957 provisional pattern*.

Essential amino acids	Mg/gm N	
Isoleucine	270	
Leucine	306	
Lysine	270	
Phenylalanine		180
Tyrosine		180
Total aromatic amino acids	360	
Methionine		144
Cystine		126
Total sulfur amino acids	270	
Threonine	180	
Tryptophan	90	
Valine	270	

* From Table 3, p 26, Protein Requirements, FAO, 1957.

Table 2. The pattern of essential amino acids in egg protein*.

Essential amino acids	Mg/gm total N	
Isoleucine		415
Leucine		553
Lysine		403
Phenylalanine		365
Tyrosine		262
Total aromatic amino acids	627	
Methionine		197
Cystine		149
Total sulfur amino acids	346	
Threonine	317	
Tryptophan	100	
Valine	454	

* From Table 6, p 36, part B, Protein Requirements, FAO/WHO, 1965.

IV. RESULTS AND DISCUSSION

This experiment was conducted at the seed technology laboratory of the American University of Beirut, to study some biochemical changes that take place during seed development of chick peas. The changes in moisture content, protein, fat, fibre, ash, and nitrogen-free-extract were studied on the seeds harvested from the field crop, while the changes in the mineral content were studied on the seeds harvested from the sand culture crop.

Changes in Chemical Composition

Proximate composition

Changes in moisture, protein, fat, fibre, ash, and nitrogen-free-extract during the five stages of seed development are shown in Table 3. The moisture percentage decreased steadily from 69.83 at the immature stage, 14 days after flowering to 12.52 at the mature stage, 42 days after flowering. All other constituents including protein, fat, fibre, ash, and nitrogen-free-extract increased with maturity of the seed. The results found here for protein, fat, fibre, ash, and nitrogen-free-extract conform to those reported by Evans (15), Bressani and Conde (7), and Verma *et al.* (42) for maize and Bengal gram at different stages

Table 3. Changes in the chemical composition of chick peas at different stages of seed development.

Stages of harvest	Moisture %	Protein %	Fat %	Fibre %	Ash %	NFE %
First stage	69.83	7.01	0.91	1.28	1.47	19.50
Second stage	61.12	7.56	2.73	1.80	1.33	25.46
Third stage	46.05	10.15	3.86	2.68	1.72	35.54
Fourth stage	23.72	15.59	-*	-	-	-
Fifth stage	12.52	17.44	5.69	3.12	2.76	58.47

*-: Not determined due to lack of seed.

of seed development.

Changes in amino acids

The total and free amino acid contents at 14, 21, 28, 35, and 42 days after flowering are shown in Tables 4 and 5. It can be seen from Table 4 that different amino acids behaved differently during the seed development of chick peas. Lysine was very high at 14 days after flowering but suddenly dropped thereafter to a practically constant value. On the other hand, histidine and cystine were very low at the immature stage but afterwards increased rather steadily up to maturity. Arginine, threonine, serine, proline, glycine, isoleucine, leucine, tyrosine, and phenylalanine were high at 14 and 21 days after flowering but had a dip at the 28th day and then increased again with maturity. Similarly alanine, valine, and methionine had the same trend like the above mentioned amino acids with the exception of having the dip at the 35th day instead of the 28th day after flowering. Aspartic acid and glutamic acid started high and remained rather constant but decreased when the seeds became mature, 42 days after flowering. The pattern of the essential amino acids except tryptophan is shown separately in Figure 1. All the essential amino acids studied increased in quantity towards maturity except lysine.

It can be seen in Table 5 that the free amino acids did not follow any definite trend during seed development.

Table 4. Total amino acid content (mg/g N) of chick peas at different stages of seed development.

Amino acid	Days after flowering				
	14	21	28	35	42
Lysine	496	254	256	253	252
Histidine	15	71	89	103	192
Arginine	406	474	298	308	558
Aspartic acid	445	387	355	378	206
Threonine	202	150	115	117	195
Serine	232	191	157	169	286
Glutamic acid	482	629	578	577	289
Proline	322	225	181	190	364
Glycine	170	167	133	138	228
Alanine	353	213	148	140	232
Cystine	19	38	40	39	65
Valine	303	212	165	159	265
Methionine	77	67	64	49	85
Isoleucine	189	182	134	158	242
Leucine	298	308	233	265	466
Tyrosine	135	102	92	120	199
Phenylalanine	189	242	167	204	369

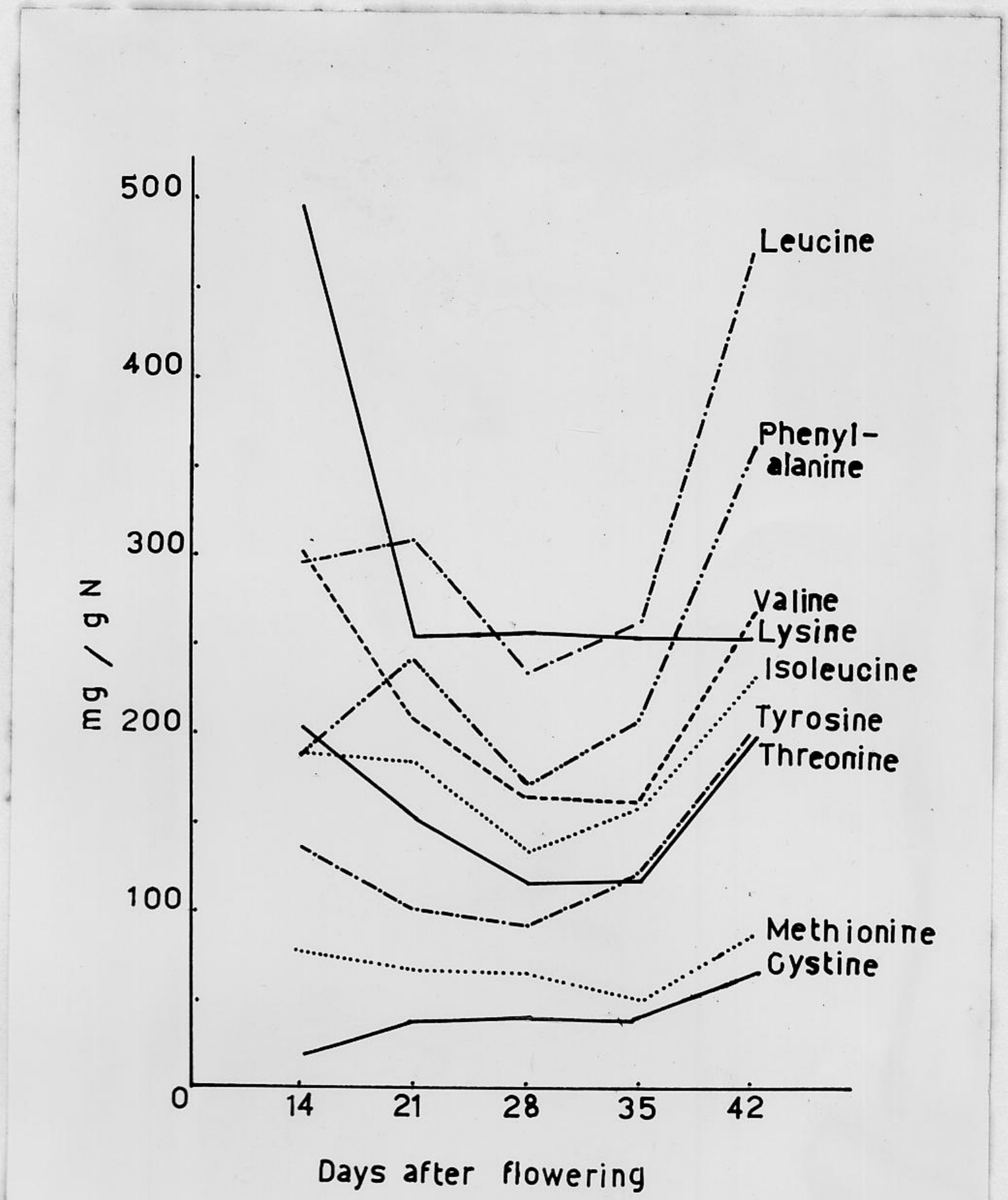

Figure 1. Pattern of change in the level of essential amino acids in chick peas during seed development.

Table 5. Free amino acid content (mg/g N) of chick peas at different stages of seed development.

Amino acid	Days after flowering				
	14	21	28	35	42
Aspartic acid	2.4	-	2.9	0.6	2.0
Threonine	5.1	-	8.9	0.5	0.6
Serine	5.5	2.1	1.0	1.2	2.7
Glutamic acid	29.7	30.2	17.7	6.1	12.8
Proline	2.1	1.8	0.4	0.8	0.6
Glycine	-	-	0.9	3.2	0.1
Alanine	-	-	5.0	0.8	1.0
Cysteic acid	-	-	0.9	0.2	0.9
Valine	-	-	2.0	-	1.3
Methionine	-	0.8	0.4	0.1	0.1
Isoleucine	7.2	2.1	0.1	0.2	0.2
Leucine	5.6	1.8	0.4	0.2	0.2
Tyrosine	0.8	-	0.6	-	-
Phenylalanine	3.1	-	0.1	-	-
Asparagine	6.3	2.5	0.4	0.8	2.8
Alpha amino-n-butyric acid	2.2	3.3	0.2	0.3	-
Norleucine	7.1	-	-	-	-
Total free amino acids	77.1	44.6	42.0	15.0	25.3

-: Not detectable

The total content of the free amino acids decreased from 77.1 mg/g N at 14 days after flowering to 25.3 mg/g N at seed maturity, 42 days after flowering. The quantity of the free amino acids of chick peas at the five stages of seed development was much smaller than that of the total amino acids (Table 5). This fact indicates that almost all the amino acids in the seeds of chick peas and at different stages of development are found in the form of protein (28). The fluctuation in the amino acid content throughout seed development was probably due to transamination and other physiological and biochemical processes in amino acid synthesis.

Protein quality

The nutritional value of a protein is related to its content of essential amino acids. The relative lack or abundance of these amino acids in a diet will determine whether a protein is good or poor for growth and tissue repairs. The protein quality scores of chick peas at different stages of seed development are shown in Tables 6, 7, and 8.

The FAO 1957 score pattern (Table 6) indicates the protein quality values on the basis of the actual amounts of the essential amino acids in the seeds as compared to the hypothetical amounts of the FAO 1957 pattern (1). It can be seen from Table 6 that the score of isoleucine

Table 6. Protein quality scores of chick peas at different stages of seed development (FAO 1957 scoring pattern).

Essential amino acid	Days after flowering				
	14	21	28	35	42
Isoleucine	70	67	49	58	89
Leucine	97	101	76	86	152
Lysine	184	94	95	94	93
Phenylalanine	105	134	93	113	205
Tyrosine	75	56	51	66	110
Total aromatic amino acids	90	95	72	90	158
Methionine	55	46	44	34	59
Cystine	15	30	32	31	52
Total sulfur amino acids	35	39	42	33	55
Threonine	112	83	63	64	108
Valine	112	78	61	59	98

is inadequate throughout the development stages of chick peas. However, the score value increases at the time of maturity. The same is true for the sulfur amino acids, methionine, and cystine, but the values of cystine are lower than those of methionine. Leucine is adequate at 21 and 42 days after flowering but is limiting at 14, 28, and 35 days after flowering. Lysine attains the score of 184 at the 14th day after flowering, but decreases suddenly and levels off to a value slightly below 100. Phenylalanine is only limiting at 28 days after flowering, while the other aromatic amino acid, tyrosine, is only adequate at the time of maturity, 42 days after flowering. Threonine and valine are more limiting at 21, 28, and 35 days after flowering and adequate at 14 and 42 days after flowering. However, at all stages of development the sulfur amino acids were the most limiting among the essential amino acids determined. The essential amino acid tryptophan was not determined in this study due to shortage of seed material.

Similarly the 1965 egg score pattern (Table 7) shows the protein quality values on the basis of the actual amounts of the essential amino acids in the seeds as compared to those of the egg (2).

According to the FAO/WHO 1965 scoring pattern the score values are adequate only in case of lysine and phenylalanine at 14 and 42 days after flowering, respectively. All the other essential amino acids at

Table 7. Protein quality scores of chick peas at different stages of seed development (FAO/WHO 1965).

Essential amino acid	Days after flowering				
	14	21	28	35	42
Isoleucine	45	44	32	38	58
Leucine	54	55	42	48	84
Lysine	123	63	63	62	62
Phenylalanine	52	66	46	55	101
Tyrosine	52	39	35	46	76
Total aromatic amino acids	52	55	57	52	91
Methionine	39	34	32	25	43
Cystine	13	25	27	26	44
Total sulfur amino acids	28	31	33	25	43
Threonine	64	47	36	37	62
Valine	67	47	36	35	58

all the stages of development were limiting.

It can be seen from Tables 6 and 7 that the score values are lower in case of the FAO/WHO 1965 scoring pattern compared to those of the FAO 1957 scoring pattern. It seems that the FAO 1957 scoring pattern over-estimates the quality of the protein when compared to the FAO/WHO 1965 pattern. Hence, the latter may be considered a more dependable pattern to be used (2).

The data in Table 8 show the final picture of protein quality based on the FAO 1957 pattern, and the FAO/WHO 1965 pattern. It can be seen that during all the stages of seed development, the sulfur amino acids, methionine and cystine, were found to be the most limiting by the 1957 scoring pattern. According to the 1965 FAO/WHO pattern the sulfur amino acids were limiting at 14, 21, 35, and 42 days after flowering, and isoleucine was found to be the most limiting, 28 days after flowering. Although isoleucine was found to be limiting at the 3rd stage of seed development, yet the difference in the score value of sulfur amino acids and isoleucine was negligible (Table 7). Hence, practically the sulfur amino acids were the most limiting at all stages of seed development of chick peas.

Changes in mineral content

The changes in the amounts of macro- and micro-elements during seed development of chick peas are shown

Table 8. Protein quality of chick peas at different stages of seed development.

Protein score based on	Days after flowering				
	14	21	28	35	42
1957 FAO pattern	35(S)	39(S)	42(S)	33(S)	55(S)
1965 Egg's pattern	28(S)	31(S)	32(I)	25(S)	43(S)

(S): Limiting sulfur amino acids.
(I): Limiting isoleucine amino acid.

in Figures 2 and 3, respectively. Since the values are reported in mg per 100 g dry matter, therefore, any change in mineral content is relative to the accumulation of dry matter during seed development and maturation. Each of the macro-elements, calcium, magnesium, sodium, and potassium and the micro-elements, iron, zinc, manganese, and copper appeared to be at the highest level 14 days after flowering, and then started to decrease later on towards maturity. The pattern of decrease varied with different elements. The divalent elements calcium and magnesium showed similar fluctuation; decreased from stage one to stage two, increased towards stage three, and later on decreased to the same value at stage 4, 42 days after flowering. In reality, minerals that accumulate in the seed do not leach out afterwards. A decrease in the mineral content indicates its relative change to the rapidly accumulating dry matter, and it means that the mineral content is either set at a constant level or that it is increasing at a relatively lower rate than dry matter. On the other hand, an increase in mineral content indicates a relatively rapid accumulation of minerals over dry matter. The results obtained here for iron are similar to those reported by Verma *et al.* (42) on Bengal gram seed (*Cicer arietinum* L.), but are contrary to those reported for calcium.

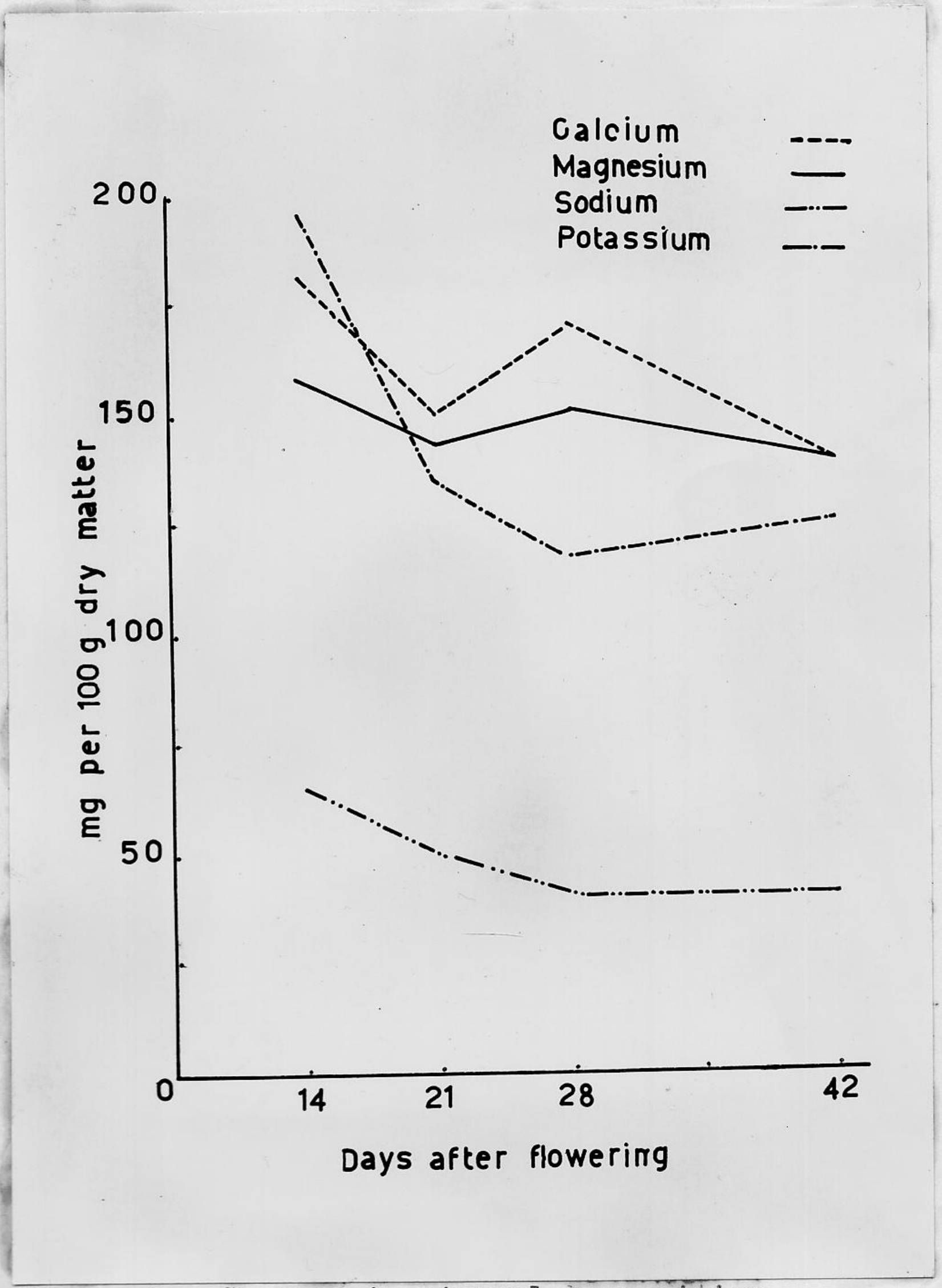

Figure 2. Changes in mineral composition of chick peas during seed development - macro-elements.

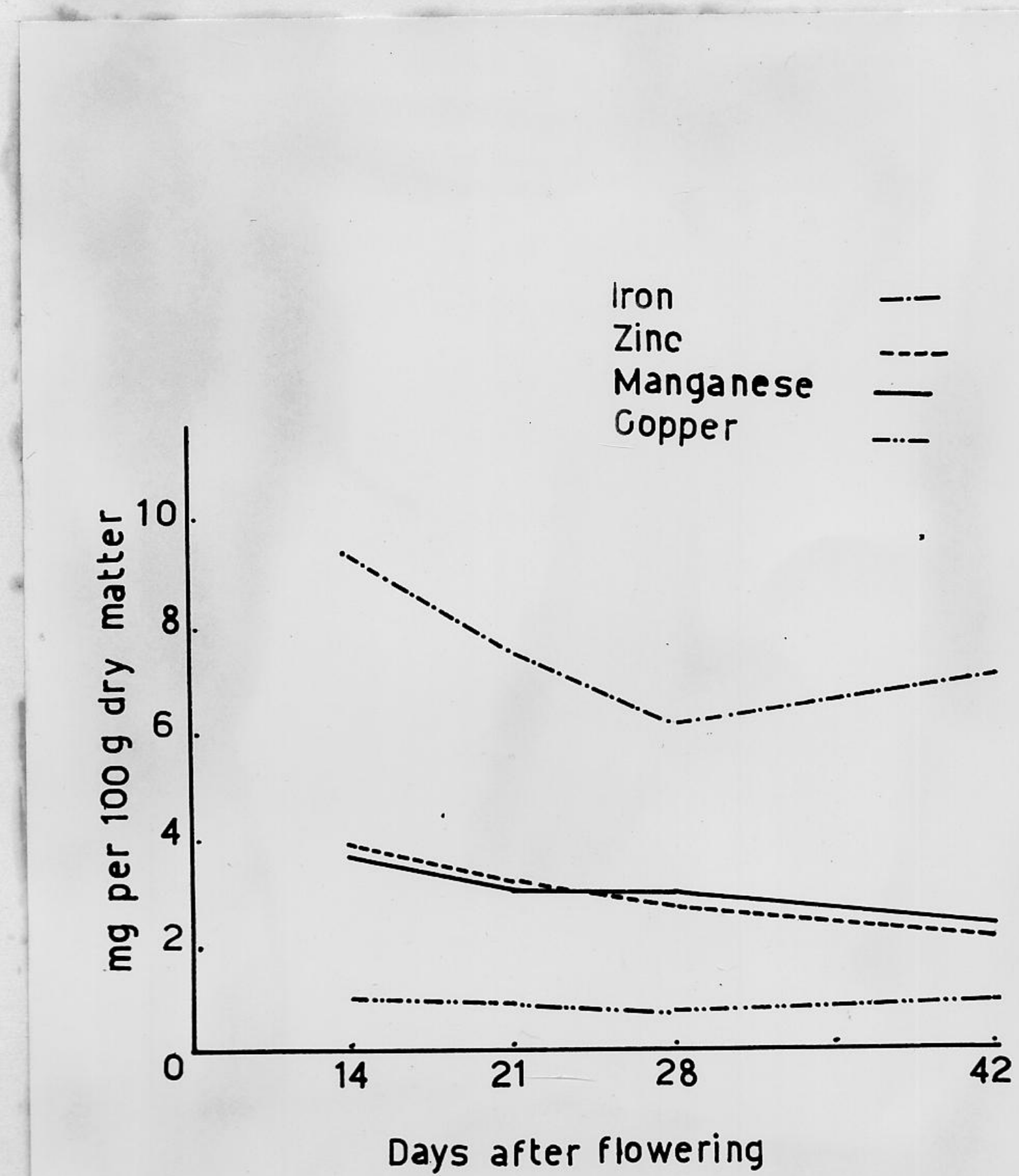

Figure 3. Changes in mineral composition of chick peas during seed development - micro-elements.

V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Summary

A biochemical study was conducted in the seed technology laboratory of the American University of Beirut, Lebanon, to study the changes of moisture content, protein, fat, fibre, ash, nitrogen-free-extract, total and free amino acids, and mineral composition in chick peas at different stages of seed development.

Constituents like protein, fat, fibre, ash, and nitrogen-free-extract increased with the maturity of the seed, only the moisture percentage decreased as the seeds of chick peas advanced in age.

Different trends in the changes of amino acid contents were observed during the developmental period of the seeds of chick peas in that, different amino acids behaved differently. All the essential amino acids studied increased in quantity towards maturity except lysine which was very high two weeks after flowering but suddenly dropped thereafter to a practically constant value. According to the FAO 1957 and the FAO/WHO 1965 provisional patterns the sulfur amino acids were found to be the most limiting ones during the entire period of seed development. The contents

of the free amino acids decreased from 77.1 mg/g N two weeks after flowering to 25.3 mg/g N at seed maturity. The quantity of the free amino acids at each stage of development was much smaller than that of the total amino acids at the corresponding stages.

The minerals calcium, magnesium, sodium, potassium, iron, zinc, manganese, and copper showed a decrease in their amounts relative to the accumulation of dry matter during seed development and maturation. Since the mineral content is reported in mg per 100 g dry matter, therefore, a decrease in the amount means that the mineral content is either set at a constant level or that it is increasing at a relatively lower rate than the dry matter.

Conclusions

The following conclusions may be drawn from the present study.

1. Almost all the amino acids in the seeds of chick peas at different stages of development were found in the form of proteins.

2. The sulfur-containing amino acids, methionine and cystine, were found to be the most limiting amino acids at all stages of seed development.

3. The protein quality or the nutritional value of chick peas was improved when the seeds became mature.

Recommendations

In the light of the limited results obtained in this study, it is suggested that more work should be done on this problem particularly on the level of non-protein nitrogen, tryptophan, carotene, and mineral contents in mg/100 seed before any final recommendations can be made.

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