Food Chemistry **19** (1986) 197–211

In Vitro Digestibility, Physico-Chemical and Functional Properties of Apricot Kernel Proteins

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(Received: 17 July, 1985)

ABSTRACT

The chemical composition of apricot kernel flour and protein isolates was determined. The flour contained about 48% protein, mainly albumins. The solubility at different pH values showed a single isoelectric point at pH 4 and the solubility increased in both acidic and alkaline pH. The digestibility of the protein was high in the pepsin–pancreatin system and quite low when pepsin or trypsin was used.

The ultraviolet absorption spectrum of the proteins of both flour and protein isolates was typical of those of other vegetable proteins. The polyacrylamide gel electrophoresis pattern of apricot kernel proteins consisted of four major protein fractions. Water and fat absorption and emulsification capacity of flour and protein isolates were quite comparable with that of soybean. Detoxified apricot kernel flour and protein isolates appear to be good sources of protein for food products.

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Food Chemistry 0308-8146/86/$03·50 © Elsevier Applied Science Publishers Ltd, England, 1986. Printed in Great Britain

INTRODUCTION

Apricot, *Prunus armeniaca*, is the most delicious stone fruit consumed during the summer season in Egypt. It is used fresh or processed as apricot juice, nectar, jam and sheets (*kamruldin*). There are several literature reports about the amino acid pattern of its flesh (Hegazi & Salem, 1972), the chemical and physical properties of apricot kernel and oil (Hallabo *et al.*, 1975), biological evaluation of apricot kernel cake (Khairy *et al.*, 1975) and apricot kernel protein (Moussa & Abo-Bakr, 1980).

The amount of apricot pits remaining after processing is quite large. It has been utilized in Germany and the United States of America to produce fixed oil and bitter almond oil (Cruess, 1958). Apricot kernel cake contains 41·5% total protein and could be used as a good source of protein except for the presence of a toxic substance, amygdalin, which is known to be lethal to humans if the consumed dose reaches 1·71 g (Jamieson, 1943). The toxic amygdalin content of cultivated Egyptian apricot kernels is 5·7% according to the report of Khairy *et al.* (1975).

The study described in this paper was conducted with a view to the removal of the toxic material from apricot kernels. The physico-chemical properties, digestibility by different enzyme systems and functional properties of the amygdalin-free apricot kernel flour and protein isolates were studied.

MATERIALS AND METHODS

Materials

Apricot fruits (*Prunus armeniaca*) were obtained from the local market of Alexandria City, Egypt, during the summer season of 1984.

Preparation of apricot kernels
The pits were removed from the fruits by hand, washed with water and air dried at room temperature for 3 months before removing the kernels by manual cracking. The kernel percentage was calculated (31·3 ± 3·8%). The kernels were then ground in a laboratory mill to pass through a 20 mesh sieve and stored at 0°C to be used for detoxification treatment.

Detoxification of apricot kernels
The method of Khairy *et al.* (1975) for removing the toxic substances from apricot kernels was followed with a slight modification. The full fat apricot kernel grits were soaked in water (1:12 w/v ratio) at 40°C overnight. The whole slurry was dried at 40°C overnight in a forced draught air oven.

After drying, fats and lipids were removed by repeated extraction with hexane. The defatted meal was air dried at room temperature (~25°C) and ground to pass through a 60 mesh (British Standard screen) sieve. The apricot kernel flour was kept at 4°C and used for analysis.

Preparation of apricot kernel protein isolate
The detoxified apricot kernel flour was extracted with water at pH 12 using a flour to solvent ratio of 1:10 and stirring for 1 h at room temperature. The pH was adjusted by the addition of 0·5M NaOH solution. The suspension was centrifuged at 1650 × *g* for 20 min and the pH of the clear supernatant was adjusted to 4 by the addition of 0·5M HCl solution. The whole mixture was kept at 4°C for 1 h for a complete precipitation of the protein and then removed by centrifugation. It was washed twice with acidified distilled water and twice with 70% ethyl alcohol. The isolated protein was dried in a forced draught air oven at 40°C overnight, kept at 4°C and used for analysis.

Analytical methods

Moisture, total protein, crude lipid and fibre, ash and hydrocyanic acid contents were determined according to the methods of the AOAC (1975). The protein content was calculated by using the factor 6·25. The total sugars were determined by the method of Dubois *et al.* (1956). Glucose was used to prepare the standard curve. Sodium chloride content was estimated by Mohr's method as described by Vogel (1961).

Nitrogen-pH solubility profile
One gram of flour was dispersed in 50 ml of distilled water and the pH adjusted in the range of 1 to 12 by 1·0M HCl or NaOH. The solution was shaken mechanically at room temperature (~25°C) for 1 h. The soluble nitrogen was separated by centrifugation at 1260 × *g* for 30 min. The clear supernatant was used for nitrogen determination by the Kjeldahl method.

Nitrogen solubility index in different solvents

This was estimated in water at pH values 7 and 12, 0·02M NaOH and 5% NaCl, as described by Rahma & Narasinga Rao (1979). Defatted flour and protein isolates were used for these determinations.

Fractionation of total protein on the basis of solubility (Osborne, 1924 classification)

One gram of flour was extracted twice with 50 ml of distilled water for 1 h at room temperature using a mechanical shaker. The extract was centrifuged at 1650 × *g* for 20 min and the clear supernatant was used for protein determination. The residue was consequently used for extraction with 1 M NaCl, 70% ethyl alcohol and 0·2 M NaOH as in water. The supernatant of each extract was collected separately and used for protein estimation. The remaining residue, after these successive extractions, was quantitatively transferred into Kjeldahl flasks and digested with conc. H_2SO_4 to determine the protein content.

Absorption spectrum

The ultraviolet (UV) absorption spectrum of the proteins of apricot kernel flour and protein isolate in 1 M NaCl solution was recorded at room temperature (~25°C) in a Perkin-Elmer double-beam recording spectrophotometer 124, in the range 240–300 nm. Protein solutions of approximately 0·1% (w/v) were used.

Polyacrylamide Gel Electrophoresis (PAGE)

This was carried out according to the procedures of Koenig *et al.* (1970) and Stegemann *et al.* (1979) using 7·5% polyacrylamide gels incorporating sodium dodecyl sulphate (SDS). Tris-borate buffer (0·125M, pH 7·1) containing 0·1% SDS was used.

Preparation of the protein sample for SDS-PAGE

Extracts from the samples were boiled for 3 min with 4% (w/v) SDS and 2% (v/v) β-mercaptoethanol (ME) in water (1:1 v/v) giving a final concentration of 2% SDS and 1% ME in the samples. A few drops of amide black (1% w/v) solution in distilled water as front indicator and sucrose were added to the samples before application.

A volume of 20 μl of the extracts was loaded and electrophoresis was performed for 2 h at a constant voltage (400 V). The gels were stained overnight with a staining solution of 0·1% (w/v) Coomassie Brilliant

Blue–R 250 in a mixture of trichloroacetic acid (TCA), methanol, acetic acid and water (3:20:7:80, w/v/v/v), then destained with a solution containing methanol, acetic acid and water (50:75:875, v/v/v).

Digestibility studies of apricot kernel flour and protein isolate
The *in vitro* digestibility index by the pepsin–pancreatin system was determined according to the procedure of Akeson & Stahmann (1964). Digestibility by the other different proteolytic enzymes in a single system was also performed as described below.

Pepsin digestibility. This was determined by incubating 0·5 g each of defatted apricot kernel flour and protein isolate with 12·5 mg of pepsin enzyme in 15 ml of 0·1 M HCl for 24 h at 37 °C in an incubator shaker. At the end of the digestion period 15 ml of 10 % TCA was added to terminate the enzyme reaction. The soluble nitrogen in the supernatant was determined by the Kjeldahl method. A sample blank was conducted under the same conditions and casein, as a standard protein, used for comparison (Moussa & Abo-Bakr, 1980).

Trypsin digestibility. Four milligrams of trypsin enzyme (Serva, from bovine pancreas 40 U/mg, 2 × cryst.) in 7·5 ml of 0·1 M sodium phosphate buffer of pH 8·0 was added to 100 mg of flour or protein isolate and incubated in a shaker incubator for 24 h at 37 °C. The reaction was terminated and the digestibility determined as described for pepsin digestibility.

Pancreatin digestibility. Pancreatin digestibility was estimated using pancreatin (Sigma, porcine pancreas. Activity equivalent to 4 × NF specification) as described for trypsin digestibility.

Functional properties

Water absorption capacity
This was measured by the method of Sosulski (1962). The values are expressed as grams of water absorbed per 100 g of flour or protein isolates.

Oil absorption capacity
The method of Sosulski *et al.* (1976) was used to measure the oil absorption capacity of apricot kernel flour and protein isolate using

refined corn oil. The oil absorption capacity is expressed as millilitres of oil bound per 100 g of sample.

Emulsification properties
Emulsification capacity and emulsion stability were measured according to the procedure of Yasumatsu *et al.* (1972) using 1 % protein solution and refined corn oil.

Foaming properties
The method of Huffmann *et al.* (1975) was followed. The measurements were made at room temperature (~25 °C) using 1 % protein solution. Foaming capacity is expressed as the percentage increase in the volume after 30 s. and foam stability is expressed as the foam volume determined after 10, 30, 60, 90 and 120 min of standing at room temperature.

RESULTS AND DISCUSSION

Proximate composition

The proximate chemical compositions of full fat apricot kernels, defatted flour and protein isolates are given in Table 1. Fats represent the major component in the kernel, followed by protein and total carbohydrates. Our results agree well with those reported by Dang *et al.* (1964). However, Hallabo *et al.* (1975) and Moussa & Abo-Bakr (1980) have reported a slightly higher protein content (30·2 %). The low protein content reported by us could be due to either interspecies variation or climatic conditions.

One of the major problems in utilizing apricot kernel as a feed or food is the presence of a poisonous cyanogenic glucoside compound, amygdalin, which, after hydrolysis, gives hydrocyanic acid (Liener, 1966). Data in Table 1 show that the apricot kernel has 0·15 % hydrocyanic acid. This value is within the range of 0·01 % to 0·18 % reported by Stoewsand *et al.* (1975) and in good agreement with the value of 0·17 % reported by Hallabo *et al.* (1975).

Rieders (1965) has reported that 2–4 mg of cyanide salt per kilogram of body weight is lethal to humans. According to the data in Table 1, apricot kernels must be detoxified before use either for feed or food purposes.

Removal of toxic compounds and fats from apricot kernel resulted in a considerable increase in the protein content (50·9 %). The increase in the

TABLE 1
Proximate Chemical Compositions of Full Fat Apricot Kernels, Defatted Flour and Protein Isolates[a]

Constituents (%)	*Samples*		
	Whole kernels	*Defatted flour*	*Protein isolates*
Total protein, N × 6·25	24·1 ± 1·6	48·1 ± 1·5	90·8 ± 1·1
Ether extract	50·9 ± 2·2	3·80 ± 1·2	0·80 ± 0·8
Crude fiber	2·40 ± 1·3	4·50 ± 1·6	ND
Total ash	2·20 ± 0·2	4·10 ± 0·4	6·80[b] ± 0·8
Total sugars (as glucose)	4·10 ± 0·5	7·76 ± 0·4	1·60 ± 0·2
Total carbohydrates excluding sugars (by difference)	16·2 ± 2·1	31·7 ± 2·2	ND
Hydrocyanic acid	0·15 ± 0·04	0·0	0·0

Moisture content values were 4·36 ± 0·2, 10·27 ± 0·4 and 20·60 ± 0·4 % for whole kernels, defatted flour and protein isolates, respectively.
[a] Average of three determinations and expressed on a dry weight basis.
[b] Including sodium chloride.
ND Not determined.

other constituents after defatting was expected. The protein isolate of apricot kernel has 90·8 % protein and the increase in ash content was due to the formation of sodium chloride during the precipitation of the protein. From the foregoing data, apricot kernel flour free from toxic substances, and its protein isolate, are a potential source of protein.

Classification of apricot kernel proteins according to the method of Osborne (1924) is given in Table 2. Albumins represent the major protein

TABLE 2
Protein Fractions of Apricot Kernel Flour[a]

Protein fraction	%
Albumins	84·7 ± 2·30
Globulins	7·65 ± 1·50
Prolamins	1·17 ± 0·70
Glutelins	3·45 ± 0·50
Non-protein nitrogen	1·17 ± 0·13
Residual proteins	1·85 ± 0·25

[a] Average of three determinations.

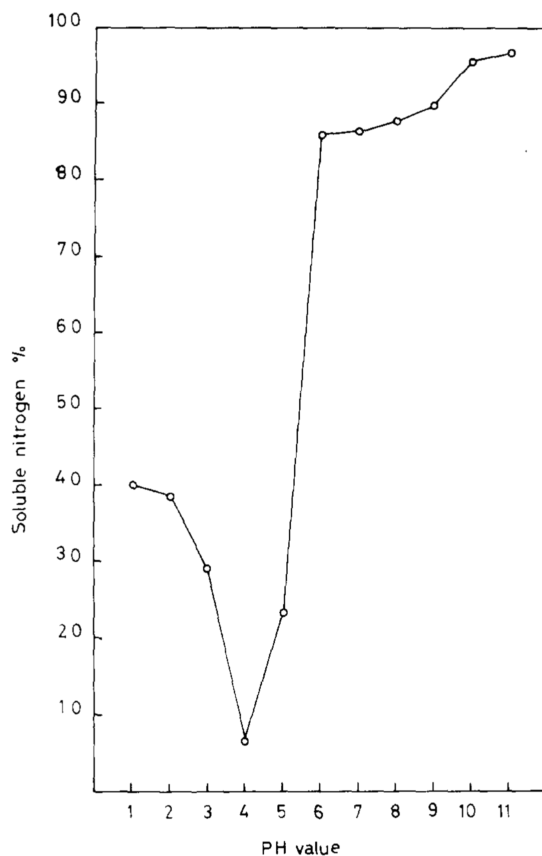

Fig. 1. Nitrogen solubility index of apricot kernel flour protein at different pHs.

fraction (84·7%). Moussa & Abo-Bakr (1980) have reported a similar value for apricot kernel flour protein. The other fractions were minor. Globulins and glutelins were 7·65% and 3·45%, respectively. These data indicate that apricot kernel has a protein of low molecular weight which could be functional.

Adele (1975) has reported that the proteins present in seeds are of two types—metabolic proteins which are normally of low molecular weight and storage proteins, mainly globulins. According to this criterion, apricot kernel contains metabolic protein (both enzymatic and structural proteins) as a major protein component.

The nitrogen solubility index of apricot kernel flour is illustrated in Fig. 1. The solubility profile in water at different pH values shows a U-shaped pattern, suggesting the existence of only one isoelectric point typical of

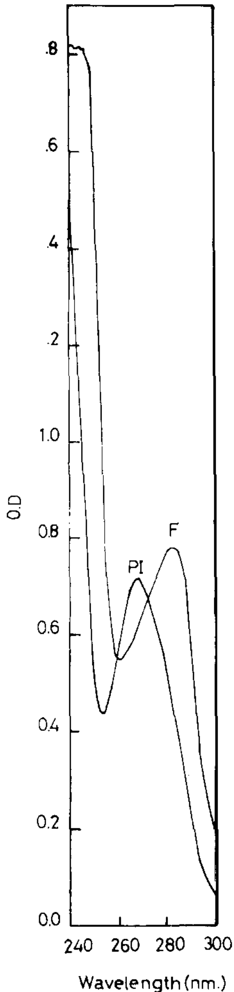

Fig. 2. Ultraviolet (UV) absorption spectrum of apricot kernel proteins in 1·0M NaCl solution.

other plant proteins such as groundnut and soybean (Fig. 1). In water, the isoelectric point of apricot kernel protein was found to be around pH 4·0. At the isoelectric point about 6·5 % of the total flour nitrogen was soluble, whereas, at the acidic and alkaline pHs, solubility was high—40·3 % and 97·1 %, respectively.

The ultraviolet absorption spectrum of apricot kernel proteins (flour and protein isolate) in 1·0M NaCl solution is given in Fig. 2. The flour showed a spectrum with a maximum at 280 nm and a minimum at 262 nm. However, the protein isolate showed different wavelength values for maximum and minimum absorption. Protein isolates had a maximum at

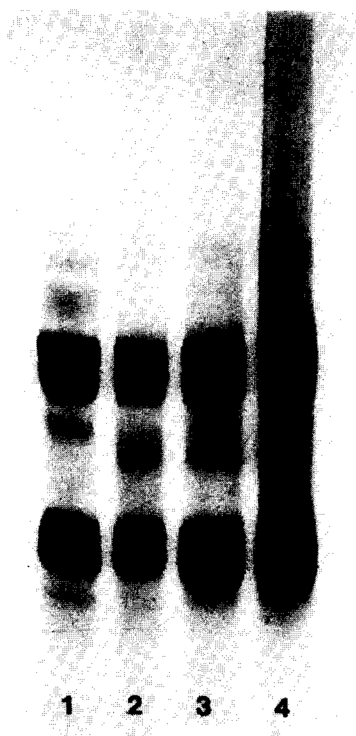

Fig. 3. SDS-polyacrylamide gel electrophoresis pattern of apricot kernel proteins in 0·125M tris-borate buffer of pH 7·1 containing 0·1 % SDS. 1,2: flour and protein isolates extracted at pH 7·7. 3,4: flour and protein isolates extracted at pH 12·0.

271 nm and a minimum at 255 nm. Both the spectra had a sharp maximum. The ratio of the absorbance at the maximum to that at the minimum was 1·42 for the flour and 1·64 for the protein isolates.

Layne (1957) has reported that proteins not conjugated with nucleic acid and other UV-absorbing impurities give a ratio of 1·5. The value for flour (1·42) was fairly close to 1·5, suggesting that both samples were free from UV-absorbing impurities. In the case of the protein isolates, there was a shift to lower wavelengths in both the maximum and minimum. Heat denaturation of guar protein (Nath, 1980) and lupin seed protein (Rahma & Narasinga Rao, 1984) gave a similar effect. The observed shift in this study may be due to the denaturation caused by the high alkaline media (pH 12) used for extracting and preparing the protein isolates.

Patterns of the separation of the proteins of apricot kernel flour and isolates by SDS-polyacrylamide gel electrophoresis are shown in Fig. 3.

Two extractions for each sample were carried out at pH 7 and 12. The pattern indicated that the subunit structure of both samples had four major fractions with almost the same molecular weight, as indicated by their migration distance. These bands are not affected by the pH of extraction. The main differences appeared between the minor bands in the upper and middle cuts, but not in the lower part, of the gel. At the same pH value of extraction, a slight difference could be seen between the patterns of flour and protein isolates. However, unexpected and close similarity was noticed in the middle region of the gel for one band in the protein isolates extracted at pH 7·0 and the extracted flour at pH 12.0. This band was missing in the flour extracted at pH 7·0 and the extract of protein isolate at pH 12·0. This may be due to the increase in flour protein solubility at the higher alkaline pHs. To the best of our knowledge the electrophoresis pattern of apricot kernel proteins has not been reported.

The *in vitro* digestibilities of apricot kernel flour and protein isolates by different enzyme systems are shown in Table 3. In general, protein isolates had a comparable digestibility to the standard protein casein in the four enzyme systems used; the flour digestibilities were low only in the single enzyme system. Pepsin digestibility was very low for casein, flour and protein isolates. The digestibility of casein and the isolates by trypsin was more than double that by pepsin. The low digestibility of flour by trypsin may be due to the presence of antitrypsin factors in the flour. This same finding was reported by Moussa & Abo-Bakr (1980) for apricot flour. Pancreatin digestibility was higher for flour and protein isolates than was pepsin and trypsin digestibility but was still low. The double enzyme

TABLE 3

In vitro Digestibility of Apricot Flour, Protein Isolates and Casein by Different Enzyme Systems[a]

Enzyme system	*Digested protein (%)*		
	Casein	*Apricot flour*	*Apricot protein isolates*
Pepsin	33·4 ± 3·1	30·6 ± 2·5	32·8 ± 2·7
Trypsin	72·8 ± 2·5	30·7 ± 3·0	66·9 ± 2·9
Pancreatin	95·9 ± 1·8	35·5 ± 2·6	95·9 ± 2·4
Pepsin-pancreatin	99·1 ± 0·3	96·4 ± 1·2	98·1 ± 1·5

[a] Average of three determinations.

TABLE 4

Nitrogen Solubility Index, Water and Oil Absorption Capacity and Emulsification Properties of Apricot Flour and Protein Isolates[a]

Functional property	*Flour*	*Protein isolates*
Protein solubility index		
pH 7 water	87·1 ± 1·5	23·7 ± 1·8
pH 12 water	97·1 ± 1·6	99·8 ± 0·2
5% NaCl in distilled water	85·9 ± 2·0	4·60 ± 1·5
0·02M NaOH in distilled water	95·1 ± 1·7	97·4 ± 1·2
Water absorption (grams of H_2O per 100 g sample)	270 ± 5·0	84·0 ± 4·0
Oil absorption (milliliters of oil per 100 g sample)	246 ± 4·0	184 ± 3·5
Emulsified oil (%)	61·0 ± 2·0	48·0 ± 1·8
Emulsion stability (%)	29·0 ± 1·0	20·0 ± 1·2
Emulsification capacity (milliliters of oil per gram of sample)	115 ± 0·3	43·0 ± 3·7

[a] Average of three measurements.

system, pepsin followed by pancreatin, gave the highest digestibility for both flour and protein isolates. The greater digestibility of protein isolates than the flour could be due to either the denaturation of the protein by several washings with ethyl alcohol and higher alkaline pH used for extraction or the heat used for drying the protein isolates. Rahma & Narasinga Rao (1984) have reported that heat denaturation of lupin seed protein increased the susceptibility towards enzyme attack. Our data for the higher digestibility of the protein isolates agree with this phenomenon.

The functional properties of apricot flour and protein isolates are given in Table 4. The protein solubility index of the flour was quite high in all the solvents used for extraction. However, the protein isolate solubility was affected markedly by the pH of the extractants. It was very low in water and 5% NaCl solution, and slightly higher than that of the flour at pH 12 and in 0·02M NaOH solution. Water and fat absorption capacities of the flour were very high compared with those of the protein isolates. The high values for the flour may be due to the presence of carbohydrates, for example, starch, which absorbs both water and oil.

Emulsification properties showed the same trends; values for flour were higher than those of protein isolates. There do not appear to be published reports concerning the functional properties of apricot kernel proteins.

Foam capacity and foaming stability of apricot flour and its protein

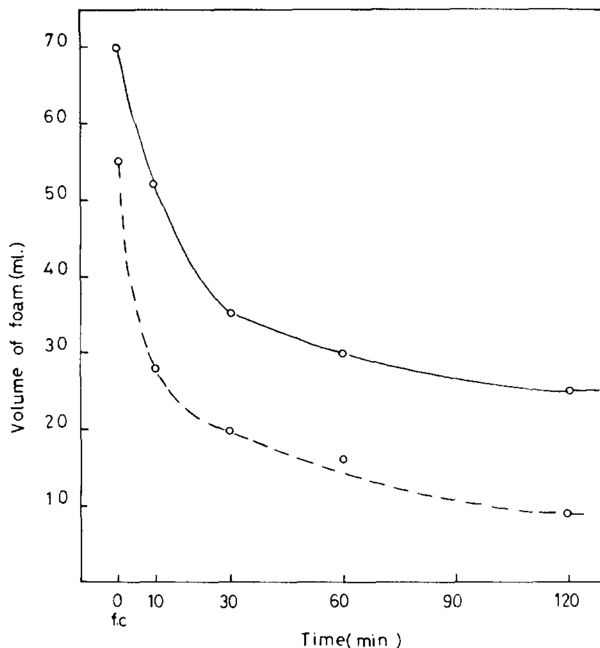

Fig. 4. Foam capacity and foaming stability of apricot kernel flour (——) and its protein isolates (- - -) at different times of standing at room temperature. (fc = foam capacity).

isolates are illustrated in Fig. 4. The foam capacity of the flour was 70 ml, compared with 55 ml for protein isolates. The same trend was observed for foaming stability. It was higher for the flour than for protein isolates. After 2 h of standing at room temperature the foaming stability of the flour was three times that of the protein isolates. The improved foaming properties of the flour may be due to the high starch content.

In general, apricot protein (especially flour) has promising functional properties which may be used to advantage in bakery products.

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