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## Functional Properties of Purified Vicilins from Cowpea (*Vigna unguiculata*) and Pea (*Pisum sativum*) and Cowpea Protein Isolate

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The major storage globulins (vicilins) of cowpea (*Vigna unguiculata*) and pea (*Pisum sativum*) seeds were purified by ammonium sulfate precipitation, and a semipurified cowpea protein isolate (CPI) was prepared by isoelectric precipitation. Some of the functional properties of these proteins, including solubility, foaming, and emulsifying capacities, were investigated and compared. The solubility of purified cowpea vicilin was reduced at pH 5.0, increasing markedly below and above this value. Pea vicilin exhibited poor solubility between pH 5.0 and pH 6.0, and CPI was little soluble in the pH range from 4.0 to 6.0. At neutral pH, the emulsifying activity indexes (EAI) of purified pea vicilin and CPI were 194 and 291 m<sup>2</sup>/g, respectively, which compare quite favorably to EAI of 110 and 133 m<sup>2</sup>/g for casein and albumin, respectively. Remarkably, purified cowpea vicilin exhibited an EAI of 490 m<sup>2</sup>/g, indicating a very high emulsifying activity. Purified cowpea and pea vicilins exhibited lower foaming capacities and foam stability indexes (FSI) than CPI. FSI values of 80 and 260 min were obtained for purified pea and cowpea vicilin, respectively, whereas a FSI value of 380 min was obtained for CPI. These results are discussed in terms of the possible utilization of purified vicilins or protein isolates from pea and cowpea in the food processing industry.

**KEYWORDS:** *Vigna unguiculata*; *Pisum sativum*; vicilin; functional properties; protein isolates

### INTRODUCTION

With the growth of the world population, there will be an increased demand for direct consumption of plant products in foods. Food ingredients enriched with vegetable proteins should at the same time be of improved nutritional value and be attractive for consumption. Proteins are often used as food ingredients for their functional properties and/or to impart certain specific characteristics to the final product. Many examples of such functional properties have been discussed (1, 2). Two of the most important functional properties of proteins relate to their ability to stabilize emulsions and foams, thus conferring desirable textural and sensorial attributes to food products. Both cases represent disperse systems in which one phase (air or oil) is dispersed throughout a continuous phase (water). Examples of emulsions are margarine and salad cream, and foams include whipped desserts and toppings. The two phases are generally immiscible and normally tend to separate in the absence of a stabilizing force. The latter can be provided by proteins, which migrate to the oil:water or air:water interface and, upon unfolding, form an interfacial layer with consequent alteration of the surface properties and stabilization of the dispersion (3).

While soybeans have historically had a competitive advantage over legume seeds, there is a need to develop other sources of concentrated plant proteins (4, 5), which ideally should be obtained from crops that are economically advantageous and widely grown in tropical countries. The cowpea (*Vigna unguiculata*) is a tropical legume whose seeds are rich in protein (about 25% in weight) (6, 7) and are widely consumed by poor populations of West Africa and of the Brazilian Northeast (8). Furthermore, it is low in antinutritional factors (9). Although the chemical compositions of cowpea (*V. unguiculata*) and pea (*Pisum sativum*) seeds have been reported in several publications (10, 11), little is known on the effects of extraction and isolation conditions on the physicochemical and functional properties of their protein isolates (12–14). One major class of seed storage proteins is the vicilins (7S globulins) (15), which are present in seeds of legumes and other plants (16). We have previously investigated the functional properties of purified pea vicilin (17). Here, we report the purification of vicilins from cowpea and pea seeds and compare some of their functional properties (solubilities, emulsifying, and foaming capacities) with those of a semipurified cowpea protein isolate (CPI).

### MATERIALS AND METHODS

**Purification of Vicilin.** Protein purification was carried out as previously described (17–19). Briefly, defatted pea and cowpea seeds

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meal was prepared, extracted with 50 mM Tris-HCl and 200 mM NaCl, pH 8.5, and centrifuged (10 000g for 30 min). Solid  $(\text{NH}_4)_2\text{SO}_4$  was slowly added to the supernatant to 45% saturation. After 30 min, the material was centrifuged at 10 000g for 30 min, and the pellet was discarded. The supernatant was brought to 75% saturation with  $(\text{NH}_4)_2\text{SO}_4$  and centrifuged. Again, the supernatant was collected, brought to 99% saturation with  $(\text{NH}_4)_2\text{SO}_4$ , and centrifuged as described above. The resulting supernatant was discarded, and the pellet was resuspended in 50 mM ammonium bicarbonate, pH 8.0, and dialyzed against the same buffer. Throughout the purification, the solutions contained a cocktail of protease inhibitors, including 0.5  $\mu\text{g/mL}$  leupeptin, 0.07  $\mu\text{g/mL}$  pepstatin, 2  $\mu\text{g/mL}$  PMSF, and 0.05  $\mu\text{g/mL}$  soybean trypsin inhibitor. Vicilin stock solutions ( $\sim 5$  mg/mL) were stored at 4 °C in 50 mM ammonium bicarbonate, pH 8.0. Protein concentration was determined according to Lowry et al. (20).

**Protein Isolate.** CPI was obtained by isoelectric precipitation from defatted cowpea meal as reported (21), with minor modifications. The defatted flour was incubated for 1 h and 30 min at 4 °C with low ionic strength buffer (50 mM Tris-HCl and 200 mM NaCl, pH 8.5) and centrifuged (10 000g for 30 min) to remove insoluble components such as fibers and pigments. The supernatant was then brought to pH 6.0 by addition of 1 M HCl and centrifuged at 10 000g, 30 min. The supernatant was then precipitated at pH 4.5 (corresponding to the isoelectric point of vicilin), and the insoluble fraction representing the CPI was collected.

**Polyacrylamide Gel Electrophoresis (PAGE).** Sodium dodecyl sulfate (SDS)-PAGE and nondenaturing PAGE were performed according to Laemmli (22). SDS-PAGE gels contained 15% (w/v) acrylamide (4% acrylamide stacking gels). Nondenaturing gels contained 6% (w/v) acrylamide (4% acrylamide stacking gels). The sample loading buffer contained 50 mM Tris-HCl (pH 6.8), 15% (v/v) glycerol, and (for SDS-PAGE only) 2% (w/v) SDS. The electrophoretic pattern was unchanged in the presence of  $\beta$ -mercaptoethanol in the loading buffer. Protein bands were stained with Coomassie Brilliant Blue R. Under nondenaturing conditions, PAGE of vicilin samples revealed a single band stained with Coomassie Blue (not shown). SDS-PAGE of purified pea vicilin revealed the presence of a major 50 kDa band plus seven posttranslational proteolytic fragments ranging from 12.5 to 33 kDa, as previously described (18, 23).

**High-Performance Liquid Chromatography (HPLC) Analysis.** Size exclusion chromatography was performed on a Shimadzu (Kyoto, Japan) HPLC apparatus equipped with a LC-10AD controller, using a precalibrated Superdex 200 column. The column was equilibrated with 50 mM Tris-HCl and 150 mM NaCl, pH 8.0. Elution was monitored by absorption at 214 nm and by fluorescence emission with excitation at 295 nm and emission at 340 nm or excitation at 275 nm and emission at 310 nm, as described in the Results. The flow rate was 0.7 mL/min. Column calibration was done with a set of proteins of known molecular weights: thyroglobulin, bovine serum albumin, ovalbumin,  $\beta$ -lactoglobulin, carbonic anhydrase, lysozyme, and aprotinin.

**Functional Properties.** The functional properties of purified vicilins and CPI were evaluated according to the following methods.

**Solubility.** Solubilities of purified vicilins and CPI were determined by a modification of the method of Coffman and Garcia (24). Protein was diluted in distilled water at a concentration of 0.5 mg/mL in a final volume of 5 mL. Then, the pH of the solution was carefully adjusted from 2 to 11 (at steps of 1 pH unit) by adding 0.1 N HCl or 0.1 N NaOH. After 60 min of stirring (with a magnetic stirrer at room temperature), the pH of the solutions was again measured and samples were centrifuged for 15 min at 34 000g. The protein concentration in the supernatants was determined according to Lowry et al. (20) and expressed as a percentage of the initial protein concentration.

**Emulsifying Properties.** Emulsions of purified vicilins or CPI were prepared as described by Dagorn-Scaviner et al. (25) by homogenizing 2 mL of *n*-dodecane with 6 mL of protein solution (1.3 mg/mL in 50 mM ammonium bicarbonate, pH 8.0) using a Polytron (Brinkmann Instruments, Westbury, NY) PT 10-35 (30 s at 20 000 rpm).

**Emulsifying Activity Index (EAI).** The EAI was estimated according to Pearce and Kinsella (26). The turbidity  $T$  ( $T = 2.303A/l$ ;  $A$ , absorbance of the emulsion;  $l$ , 1 cm, path length of the cuvette) of the emulsion, diluted 250-fold with a solution of 0.1% SDS, 0.1 M NaCl, pH 7.0,

was immediately measured at 500 nm. EAI is defined as

$$\text{EAI} = 2T/\phi C \quad (1)$$

where  $\phi$  is the volume fraction of the oil phase (here,  $\phi = 0.25$ ) and  $C$  is the protein concentration in the aqueous phase. EAI, expressed in  $\text{m}^2/\text{g}$ , is related to the stabilized interfacial area per unit weight of protein (25, 27).

**Emulsion Stability.** This was determined by following the separation of aqueous phase during aging of the emulsion (25). Immediately after preparation, the emulsion (8 mL) was poured into a graduated cylinder and destabilization was followed by plotting the volume of separated aqueous phase ( $V_t$ ) as a function of time ( $t$ ).

**Foaming Properties.** These were expressed as foaming capacity and foam stability (28). Foaming was induced by bubbling a stream of nitrogen (2.5 mL/s) during 15 s through a plastic Millipore filter holder placed at the bottom of a Pyrex column containing 3 mL of protein solution (1.0 mg/mL in 50 mM ammonium bicarbonate, pH 8.0). The conductivity of the resulting foam was recorded using a conductivity meter (Cole-Parmer Instrument Co., Chicago, IL). Foaming capacity was evaluated by the initial conductivity ( $C_i$ ,  $\mu\text{S}/\text{cm}$ ). Foam stability was expressed as (i)  $t_{1/2}$ , the time necessary to obtain a conductivity of  $C_i/2$  measured on the experimental curve, and (ii) the foam stability index (FSI), defined by Kato et al. (29) as

$$\text{FSI} = C_0 \Delta t / \Delta C \text{ (min)} \quad (2)$$

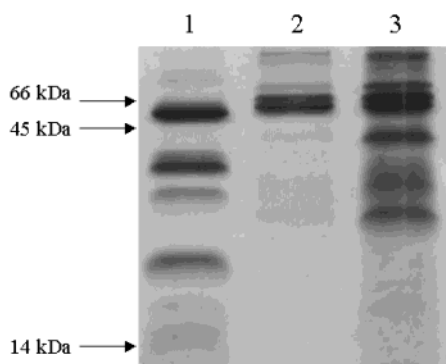
where  $C_0$  is the conductivity at zero time obtained by extrapolation of the linear part of the  $C$  vs  $t$  plot and  $\Delta C/\Delta t$  is the slope of this linear region. The value of  $t_{1/2}$  is related to drainage of liquid that occurs just after foam formation, while FSI describes the stability of the foam when most of the drainage has occurred and is mostly related to coalescence (27, 29).

**Amino Acid Determination.** Amino acid analyses were performed on a Biochrom 20 (Pharmacia Biotech, Uppsala, Sweden) automatic amino acid analyzer based on the methodology described by Spackman et al. (30). Protein samples were vapor hydrolyzed in a Pico Tag workstation (Waters Corporation, U.S.A.) by standard hydrolysis, after saturation of the atmosphere with nitrogen, using 200  $\mu\text{L}$  of 5.7 N HCl for 24 h at 108 °C. Maintenance of an inert nitrogen atmosphere during the hydrolysis is important to minimize or prevent the oxidation of labile sulfur-containing amino acids. The amino acid composition was calculated considering the highest value obtained for each amino acid, except for tryptophan content, which was determined by the ultraviolet molar absorption coefficient of each protein sample (31).

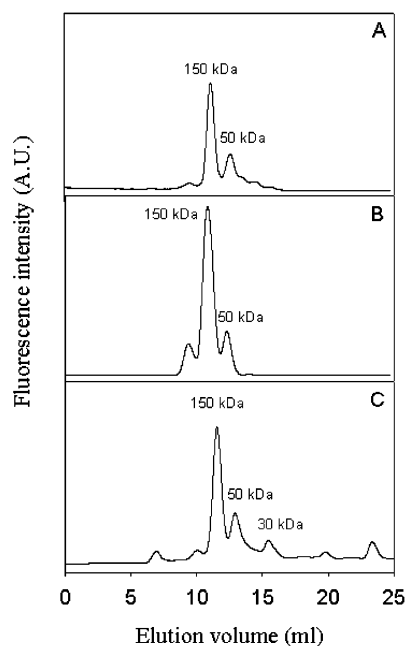
## RESULTS AND DISCUSSION

Protein quality is often affected by the presence of anti-nutritional factors such as protease inhibitors, lectins, tannins, stachyose, and raffinose, which may interact with the intestinal tract and reduce protein digestibility and amino acid absorption (32, 33). Cowpea and pea seeds contain antinutritional factors that can be eliminated or inactivated to a large extent by appropriate heating and processing during food preparation (33–35). Upon processing, dehulling has been reported to reduce the amount of stachyose and raffinose in cowpeas, while almost eliminating its tannin content (34). Wet milling and processing techniques employed during protein concentration and isolation are known to be effective in the detoxification of seed materials (35). Most of these compounds inhibit digestive enzymes or react with essential amino acids, limiting the application of the seeds in many food products. In the present work, we have obtained both purified proteins and semipurified protein isolates from cowpea and pea seeds and carried out an investigation of some of their functional properties of potential interest to the food processing industry.

**Physicochemical Properties.** Purified vicilins and CPI were routinely examined by SDS-PAGE under denaturing conditions (Figure 1). The vicilins and CPI had main protein bands of 50



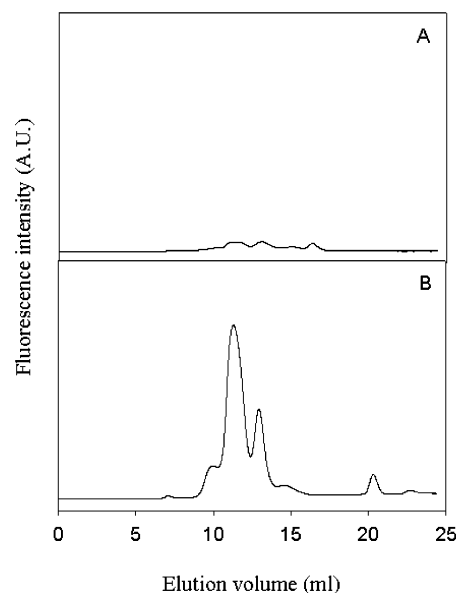
**Figure 1.** SDS-PAGE analysis of the protein preparations. Samples were prepared and analyzed as described in the Materials and Methods. Lane 1, purified pea vicilin; lane 2, purified cowpea vicilin; lane 3, CPI. Forty micrograms of total protein was applied in each lane.



**Figure 2.** Size exclusion HPLC analysis of purified pea and cowpea vicilins and CPI. Vicilins or CPI samples were applied onto a Superdex 200 column and chromatographed as described in the Materials and Methods. Total protein applied per run was 100  $\mu$ g. (A–C) Purified vicilin from pea, cowpea, and CPI, respectively. Elution was monitored by intrinsic fluorescence (excitation at 275 nm and emission at 310 nm). The flow rate was 0.7 mL/min.

and 52 kDa, respectively. These are in the typical molecular mass range of 7S storage proteins, in agreement with data previously reported by several groups (36–38). In addition to the main 50 kDa band, preparations of purified pea vicilin contained additional bands of 38 and 33 kDa and minor bands of 23 kDa. All of these bands have previously been shown to result from posttranslational proteolytic processing of the intact 50 kDa vicilin subunit (18). Interestingly, purified cowpea vicilin preparations consisted almost exclusively of a doublet of bands around 52 kDa and CPI contained polypeptides ranging from 30 to 52 kDa. It is interesting to note that purified cowpea vicilin preparations were essentially devoid of lower molecular mass bands, suggesting that proteolysis of storage proteins is lower in cowpea seeds than in pea seeds.

**HPLC Analysis of Purified Vicilins and CPI.** Figure 2 shows size exclusion chromatography profiles of purified vicilin and CPI samples. For purified pea and cowpea vicilins (panels



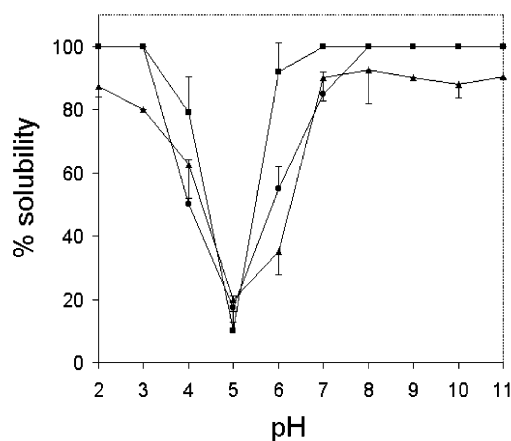
**Figure 3.** Size exclusion HPLC analysis of purified pea and cowpea vicilins. Vicilin samples were applied onto a Superdex 200 column (total protein applied per run was 100  $\mu$ g). The column was equilibrated with buffer containing 50 mM Tris-HCl and 150 mM NaCl (pH 8.0). Elution was monitored by tryptophan fluorescence detection (excitation at 295 nm and emission at 340 nm). The flow rate was 0.7 mL/min. Column calibration was done with a set of proteins of known molecular weights: thyroglobulin, bovine serum albumin, ovalbumin,  $\beta$ -lactoglobulin, carbonic anhydrase, lysozyme, and aprotinin. (A) Pea vicilin; (B) cowpea vicilin.

A and B, respectively), the protein eluted as a main peak at a volume corresponding to  $\sim$ 150 kDa in the calibrated column, followed by a smaller peak of  $\sim$ 50 kDa. Comparison with literature data reveals significant similarity in chromatographic profiles between our preparations of purified vicilins and previously reported results (18). CPI samples eluted as a major peak corresponding to  $\sim$ 150 kDa (panel C), also followed by a smaller 50 kDa fraction. Furthermore, we note that a minor peak corresponding to  $\sim$ 30 kDa was present in the CPI sample but not in the purified vicilins. The 30 kDa band in CPI likely represents a minor protein component in cowpea seeds that coprecipitates with vicilin in the isoelectric precipitation step at pH 4.5.

Interestingly, HPLC analysis also showed that contrary to pea vicilin (18), cowpea vicilin contains tryptophan residues. Figure 3 shows that when the chromatographic runs were monitored using conditions for tryptophan fluorescence detection (excitation at 295 nm and emission at 340 nm) the elution of pea vicilin could not be detected (panel A), while cowpea vicilin (panel B) clearly presented tryptophan fluorescence emission. According to Wright (39), vicilins from a number of seeds including *Phaseolus vulgaris*, *P. sativum*, and *Canavalia ensiformis* do not contain tryptophan residues in their amino acid composition. However, phaseolin from *P. vulgaris* is known to contain tryptophan. This result suggests that in terms of its nutritional value, cowpea vicilin is superior to pea vicilin.

It is also important to note that CPI (prepared by isoelectric precipitation) is considerably easier and cheaper to obtain than purified vicilins, which are obtained by successive steps of precipitation using ammonium sulfate. Furthermore, the protein yield of the protein isolate was significantly higher than the yield for purified vicilins: out of 1 g of the defatted seed flour, approximately 10 mg of purified vicilin or 120 mg of protein isolate could be routinely obtained.





**Figure 4.** pH dependence of the solubilities of purified pea and cowpea vicilins and CPI. Samples contained 0.5 mg protein/mL in distilled water at the indicated pH values. Cowpea vicilin (■), pea vicilin (▲), and CPI (●). Symbols represent means  $\pm$  SD of four determinations with different protein preparations.

**Table 1.** Emulsifying Properties of Purified Vicilins and CPI<sup>a</sup>

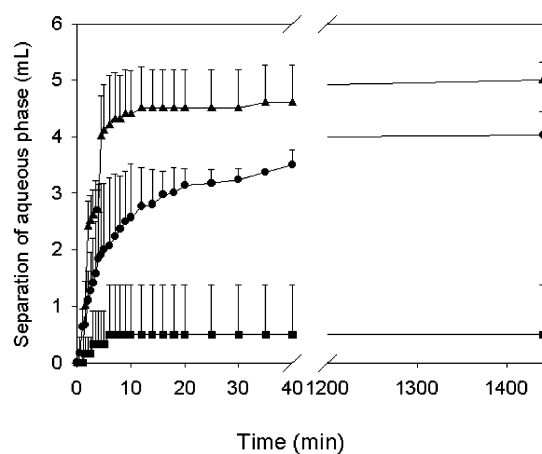
protein	EAI (m <sup>2</sup> /g)	V <sub>e</sub> (mL)	$\phi_e$
pea vicilin	194 $\pm$ 8	5.0	0.42 $\pm$ 0.06
cowpea vicilin	490 $\pm$ 55	0.5	0.21 $\pm$ 0.02
CPI	291 $\pm$ 18	4.5	0.34 $\pm$ 0.02

<sup>a</sup> Emulsions were prepared using purified pea vicilin, purified cowpea vicilin, or CPI. EAI measured at 1.3 mg protein/mL; V<sub>e</sub>, aqueous phase volume at equilibrium;  $\phi_e$ , volume fraction of oil phase. EAI and  $\phi_e$  values represent means  $\pm$  standard deviations of four independent determinations using different protein preparations.

**Functional Properties of Cowpea and Pea Proteins.** Highly soluble proteins are desired for optimal functionality in food processing applications in which emulsifying or foaming activities are required (1). Therefore, it seemed of interest to first examine the solubilities of purified vicilins and CPI.

**Solubility.** Solubilities of CPI in aqueous solution were measured and compared to the solubilities of purified vicilins. **Figure 4** shows solubilities as a function of pH in the range from 2 to 11. Cowpea vicilin was virtually insoluble at pH 5.0, but its solubility increased sharply below or above this pH value. At pH 6.0, the solubility of cowpea vicilin was >90%, and it was ~80% at pH 4.0. Pea vicilin was quite insoluble at pH 5.0–6.0 and was moderately soluble (~65%) at pH 4.0. CPI exhibited reduced solubility ( $\leq$ 50%) in the pH range from 4.0 to 6.0. These results are similar to those reported for other legume proteins (40–42). In the three cases, the results obtained can be considered satisfactory, as they indicate that these proteins can be used in the food industry in processes carried out at pH values lower or higher than their isoelectric points (~pH 5). It is important to note that all proteins investigated showed high solubilities at pH  $\geq$  7, indicating that they could easily be incorporated into products that have neutral or basic pH. Possible uses include baked products, diet drinks, and desserts (43).

**Emulsifying Activity and Emulsion Stability.** Emulsifying capacities and emulsion stabilities were determined for purified vicilins from pea and cowpea and for CPI. Emulsifying capacity was expressed in terms of the EAI, which measures the ability of a protein to help dispersion of an oil phase into an aqueous medium (25, 27). Cowpea vicilin presented a significantly higher EAI when compared with pea vicilin and CPI (**Table 1**). The three preparations presented good EAI values when compared



**Figure 5.** Kinetics of emulsion destabilization. Emulsions of *n*-dodecane in aqueous buffer were prepared as described in the Materials and Methods and the volume of separated aqueous phase was measured at different time intervals. Cowpea vicilin (■), pea vicilin (▲), and CPI (●). Symbols represent means  $\pm$  SD of four experiments using different protein preparations.

to proteins that are often considered good emulsifying standards, such as casein and albumin (EAIs = 110 and 133 m<sup>2</sup>/g, respectively; 22). Dagorn-Scaviner et al. (25) have shown that vicilin is significantly more effective than legumin in terms of its emulsifying capacity and emulsion stabilization, which may be partly due to the lower molecular weight of vicilin. In agreement with this observation, we found that purified cowpea vicilin had a much higher EAI than CPI, which may be due to the presence of other protein components in the protein isolate.

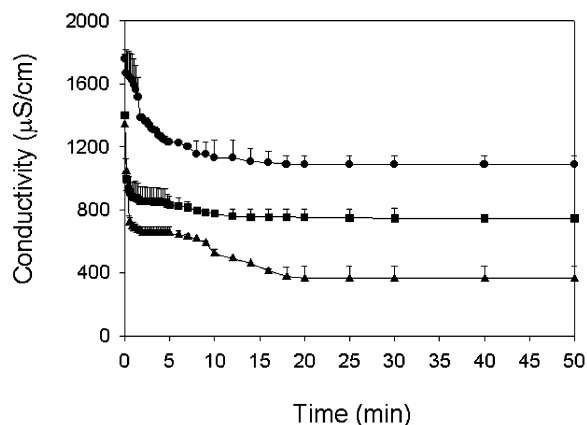
The volume of separated aqueous phase at equilibrium (V<sub>e</sub>) in the emulsion of cowpea vicilin was much smaller than the volumes obtained in the emulsions of pea vicilin and CPI (**Table 1**), which also indicated increased emulsion stability with cowpea vicilin samples. Direct measurements of emulsion stability were carried out by following the kinetics of separation of aqueous phase following vigorous stirring of the emulsion. **Figure 5** shows the kinetics obtained for purified vicilins and CPI samples. Cowpea vicilin conferred a marked stability to the emulsion, with only minor phase separation even after several weeks of incubation of the emulsion at room temperature (data not shown). Although not nearly as effective as cowpea vicilin, pea vicilin and CPI also conferred significant stability to the emulsions (**Figure 5**). Inspection of the volume fraction of the oil phase ( $\phi_e$ ) reveals an  $\phi_e$  of 0.21 for cowpea vicilin, reinforcing the conclusion that this protein exhibits excellent emulsifying capacity. These results indicate that all three protein preparations investigated (especially purified cowpea vicilin) are effective emulsifiers, making them potentially useful in applications such as the manufacture of mayonnaise, sausages, and seasonings.

**Foaming Capacity and Foam Stability.** Foaming capacity and foam stability were also compared for purified vicilins and CPI. Foaming reflects the capacity of proteins to form stable layers surrounding gas droplets in a liquid phase. Proteins with good foaming properties should be soluble in the aqueous phase, diffuse and concentrate at the air/water interface, partially unfold to form a cohesive layer around the gas bubbles, and possess sufficient viscosity and mechanical strength to prevent rupture and coalescence of the droplets (1). Foaming capacity was estimated from the value of initial conductivity (C<sub>i</sub>), measured immediately following foam formation by bubbling gas into the protein solution (29). **Table 2** shows that the initial conductivity

**Table 2.** Foaming Properties of Purified Vicilins and CPI<sup>a</sup>

protein	$C_i$ ( $\mu\text{S}/\text{cm}$ )	$t_{1/2}$ (min)	FSI (min)
pea vicilin	1338 $\pm$ 109	6 $\pm$ 1	80 $\pm$ 7
cowpea vicilin	1409 $\pm$ 132	30 $\pm$ 2	260 $\pm$ 8
CPI	1752 $\pm$ 38	50 $\pm$ 1	380 $\pm$ 11

<sup>a</sup> Foaming was induced by bubbling a stream of nitrogen in a solution containing 1.0 mg protein/mL.  $C_i$ , initial conductivity;  $t_{1/2}$ , time corresponding to  $C_i/2$ . Values represent means  $\pm$  SD of three different experiments using different protein preparations.



**Figure 6.** Kinetics of foam destabilization. Foaming of protein solutions was induced as described in the Materials and Methods, and conductivities were measured at different times after foaming. Cowpea vicilin (■), pea vicilin (▲), and CPI (●). Symbols represent means  $\pm$  SD of four experiments using different protein preparations.

was significantly higher for CPI, followed by cowpea and pea vicilin, respectively. The kinetics of foam destabilization for the three protein preparations were followed by measuring the decrease in conductivity with time as shown in **Figure 6**. The foaming properties of CPI and purified vicilins were evaluated at pH 8.0 because an increase in foam formation at alkaline pH has been described for many other protein isolates (44, 45). The high foaming capacities at slightly alkaline pH may be due to an increase in the net charge of the protein, which weakens hydrophobic interactions and increases protein flexibility, allowing them to partially unfold more effectively at the air/water interface, encapsulating air particles and increasing foam formation (41). Both cowpea and pea vicilin exhibited lower foaming capacities and stabilities when compared to CPI (**Table 2**), which may be related to their lower degree of unfolding at the air/water interface. The CPI and purified cowpea vicilin showed higher FSI (380 and 260 min, respectively) when compared to purified pea vicilin (80 min) or bovine serum albumin (FSI = 87 min; 27).

**Amino Acid Composition.** **Table 3** shows the amino acid compositions of purified cowpea vicilin and CPI. The amino acid analyses were quite similar to previously reported results for pea vicilin (27). These results are also in line with a very recent report on the composition of seeds from *V. unguiculata* and *P. vulgaris* (7). There were only slight variations in the amino acid compositions of purified vicilin and CPI. Both samples were rich in aspartic and glutamic acids. Methionine and tryptophan were limiting amino acids in both samples. The low methionine content can be overcome when legume proteins are consumed with other food ingredients containing high or moderate amounts of sulfur-containing amino acids, such as cereals proteins. These results also show that *V. unguiculata* proteins contain adequate levels of lysine, leucine, isoleucine,

**Table 3.** Amino Acid Compositions (mol %) of Purified Cowpea Vicilin and CPI

amino acid	vicilin (mol %)	CPI (mol %)	amino acid	vicilin (mol %)	CPI (mol %)
lysine	6	6	proline	5	5
leucine	9	7	tyrosine	2	3
isoleucine	5	5	glycine	5	7
threonine	4	6	serine	7	7
methionine	1	1	alanine	5	6
valine	6	6	aspartic acid	12	11
phenylalanine	6	5	glutamic acid	17	13
arginine	6	5	cysteine	0	2
histidine	3	3	tryptophan	1	2

threonine, valine, phenylalanine, arginine, and histidine, based on the FAO/WHO/UNU (46) reference pattern for infants. Interestingly, purified cowpea vicilin did not present cysteine in its composition, while the CPI presented this amino acid, likely reflecting a contribution from other protein components in the isolate. It is also important to note that although these proteins have a low content of sulfur amino acids, additional studies on rats fed experimental diets based on the CPI indicated that the protein isolate has good nutritional quality, is low in antinutritional factors, and shows no toxicity to the small intestine and pancreas (to be published elsewhere).

In conclusion, the results presented here support the potential use of both purified vicilins and CPI in the food industry. For all protein preparations investigated, the solubility was highest at acid and alkaline pH values. The emulsifying activity and emulsion stability were quite extraordinary for purified cowpea vicilin and were better than for purified pea vicilin and CPI, while the foaming capacity and stability were higher for CPI. Most essential amino acids of both purified cowpea vicilin and CPI were present in acceptable levels as compared to the FAO/WHO/UNU reference pattern for preschool children and adults.

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