Gelation Behavior of Protein Isolates Extracted from 5 Cultivars of *Pisum sativum* L.

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ABSTRACT: Protein isolates were extracted from 5 pea (*Pisum*) cultivars and their gelation behaviors were compared at pH 7.6. Gel formation and development was monitored via constant oscillation dynamic measurements. The standard heating and cooling rate was 1.0 °C/min, but samples were also heated at 0.5 °C (and cooled at 1.0 °C/min), and others were heated at 1.0 °C/min and cooled slowly at 0.2 °C/min. When heating more slowly, no changes in gel formation were detected for any of the cultivars. When cooling slowly, the cultivar Solara, with the highest legumin content, formed a stronger gel than all the other cultivars. It did the same when the thiol-blocking agent *N*-ethylmaleimide (NEM) was added to the system. This indicated that the strengthened gel system formed independently of any disulfide bonds formed by the legumin. The cultivars Supra and Classic formed stronger gels only when cooled slowly in the presence of NEM, and so disulfide bond formation in their gel systems was apparently a factor that prevented gel network strengthening. The cultivars Finale and Espace were unable to form strong and self-supporting gels. This was believed to be because of the repulsive forces on the α-subunits of vicilin, which were at their highest level in the cultivars Finale and Espace. The contribution of legumin to the pea protein isolate gels was shown to be cultivar specific and related to its disulfide bonding ability rather than the absolute amount of legumin protein present.

Keywords: *Pisum*, protein isolate composition, heat-induced gelation, legumin, vicilin

Introduction

Understanding structure-function relationships of food proteins can aid further development of their applications and enable substitution of 1 protein by another. The major leguminous plant protein that is established as a food ingredient is soybean protein and its products (protein isolates and concentrates) (Marcone and others 1998). Other legume proteins do not appear to have found a way into the spotlight. The reason why is not clear. It can be that the protein functionality is not sufficiently understood to promote their development, that the quality of their functionality is inferior relative to that of soybean, or that the functionality varies too much from 1 plant variety to another.

Pisum (pea), as a protein source, is genetically variable. Not only does the total protein content vary, so does the ratio of the 2 major globular proteins. Ratios of legumin to vicilin can be found between the extremes of 0.2 and 1.5 (Casey and others 1982). Furthermore, the specific amino acid profile of the globulin proteins varies, depending on the specific sequence of the encoding gene. As a consequence, the position and number of post-translational cleavage sites can differ from 1 protein precursor molecule to another. Post-translational proteolysis of legumin is responsible for the formation of the acidic and basic polypeptides (that can be separated under reducing conditions). Four/five acidic polypeptides (38 to 40 kDa; pI 4.5 to 5.8) and five/six basic polypeptides (19 to 22 kDa; pI 6.2 to 8.8) have been reported (Casey 1979a, 1979b; Krishna and others 1979; Matta and others 1981). Legumin molecules of about 40 kDa that are unprocessed have also been identified. The same type of genetic variability is apparent in the glycinin molecules of soybean. A study on soybean glycinin gelation even reported gel hardness to be directly proportional to the percentage of AS-III polypeptides present (the biggest of the acidic polypeptides) (Nakamura and others 1984a). Similarly, tofu made from soybeans containing the A5 polypeptide was reported to be harder and more solid-like than that prepared from cultivars that lacked the A5 polypeptide (Nishinari and others 1991). In the Vicilin gene family, 9 genes encode for the smaller protein of about 50 kDa, and 2 for the larger protein of about 70 kDa. Depending on the specific gene, the 50-kDa protein can have 2, 1, or no post-translational cleavage sites. Proteolytic processing creates six/seven small fragments between 36 and 12 kDa. In a study that separated the protein into subfractions with different small fragment compositions it was concluded that the differences in their thermal denaturation temperatures were too small to be important (O'Kane and others 2004a). No studies comparing the functionality of peas lacking 1 or more of the small fragments are known to exist. The larger protein (about 70 kDa) has been shown to be responsible for the heterogeneous gelation behavior exhibited by pea vicilins at near-neutral pH conditions (O'Kane and others 2004b), although not because of any compositional variation of this protein itself. Pea cultivars with a different total content of this protein may well exhibit different functional properties.

Understanding which, if any, of the previous factors influence the functionality of pea flours and isolates would enable cultivars to be screened and selected for given applications. This article presents the results on a study of the gelation of pea protein isolates extracted from 5 different *Pisum* cultivars. Having previously gained knowledge on the heat-induced gelation behavior of the individual pea proteins, legumin, and vicilin (O'Kane and others 2004b, 2004c), the aim was to determine whether this knowledge

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enabled the gelation behavior of the pea protein isolates to be predicted.

Materials and Methods

Pea protein isolate was obtained by isoelectric precipitation of a total protein extract of pea flour from the cultivars Solara, Supra, Classic, Finale, and Espace. All cultivars were grown under the same conditions by Cebeco Seeds (Lelystad, The Netherlands) in 1998 and harvested at the same time. The peas were subsequently air-dried. Peas were milled in a Waring commercial blender (New Hartford, Conn., U.S.A.) 2:1 (w/w) with dry ice to avoid any heat denaturation of the proteins. Salt-soluble proteins were then extracted into a 100 m*M* Tris-HCl buffer, pH 8.0, with a flour to buffer ratio 1:10 (w/v). Extraction time was 1 h at room temperature, and the extract was collected by centrifugation (11900 × *g*, 10 °C, 25 min). Isoelectric precipitation, pH 4.8, was used to isolate the globulin proteins from the total protein extract; the pH was adjusted by dropwise addition of 1 *M* HCl. Precipitated protein was left for 2 h, 4 °C before it was collected by centrifugation (11900 × *g*, 10 °C, 25 min). Washing the protein pellet with water (pellet to water ratio of 1:10) removed unwanted albumin proteins. Again the pellet was collected by centrifugation (11900 × *g*, 4 °C, 25 min), freeze-dried, and stored at –20 °C. The dried product was called pea protein isolate.

This same pellet as described previously was further treated to eventually obtain the purified proteins vicilin and legumin (that are shown on the sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE] in Figure 1). The purification method is described in detail in O'Kane and others (2004a).

Gel electrophoresis samples were prepared by mixing the protein sample at a ratio of 1:1 with sample buffer (1.4 mL distilled water, 2.0 mL 0.5 *M* Tris-HCl at pH 6.8, 2.0 mL 10% (w/v) SDS, 2.0 mL glycerol, and 0.4 mL 0.05% bromophenol blue), and heating in a boiling water bath in sealed eppendorf vials for 10 min. The protein samples were made by mixing with 75 m*M* potassium phosphate buffer, pH 7.6, for 2 h, and then spinning down to avoid insoluble material in the sample being applied on the gel; 12% polyacrylamide Tris-HCl gels were used and a volume containing 2, 5, or 10 µg of protein was loaded into each well. Low-molecular-weight protein standards, ranging from 94 to 14 kDa (Amersham Biosciences, Uppsala, Sweden) were made according to instructions, and 10 µL was applied per well when used. Gels were run at a constant 200 V. Staining was done using Coomassie Blue R-250 Bio-safe stain (Bio-Rad, Veenendaal, the Netherlands).

Estimation of protein composition of the protein isolate was determined by imaging densitometry (Bio-rad Densitometer G-710) using the gels obtained as described previously that were loaded with 2 µg and 5 µg of protein (10 µg was overloaded with poor separation of the bands). The gel loaded with 5 µg of protein is shown in the results. The percentage of legumin and vicilin, and the proportion of vicilin that was α-subunits, were calculated according to the area under the peak of staining density. The protein bands were selected on the basis of the composition of the pure proteins, which were run on the same gel. (The pure proteins were obtained from the cultivar Solara according to the method previously described in O'Kane and others 2004a.) The composition of the portion of sample soluble in potassium phosphate buffer was assumed to be the composition of the protein active in gel network formation (see results for further explanation). Results are presented as an average of the results obtained from the 2 gels.

Nitrogen content determination

The nitrogen content (%) in the protein isolates was determined using the dynamic flash combustion method (NA 2100 nitrogen and protein analyzer, CE Instruments, Milan, Italy). Triplicate samples of 5, 10, and 15 mg were used for the determination, methionine was the calibration sample, and the protein conversion factor used was 5.3. Results are presented as an average.

Minimum gelling concentration was determined by making protein isolate suspensions of 10% to 20% (w/v) concentration at pH 7.6. All samples were heated (in sealed tubes to avoid evaporation) in a boiling water bath for 30 min. Samples were cooled to room temperature for 1 h and then stored at 4 °C overnight. The next day the tubes were inverted, and the samples that did not flow after 24 h were considered to have gelled.

Small deformation rheology

Samples of the protein isolates, 18% concentration (w/v), were prepared in 75 m*M* potassium phosphate buffer and adjusted to pH 7.6 with dropwise addition of 1 *M* NaOH, and maintained at this pH throughout. Samples were mixed well for 2 h at room temperature to get a good dispersion of the isolate in the buffer. Where used, the thiol-blocking agent *N*-ethylmaleimide (NEM) was added at a concentration of 20 m*M*. Gelling was done by heating samples in a Bohlin CVO rheometer concentric cylinder (C-14). The heating profile was 45 °C to 98 °C, holding at 98 °C for 30 min, cooling to 25 °C, and holding at 25 °C for 30 min. Sample volume was 3 mL, and a few drops of vegetable oil were put on the top of the sample to prevent evaporation during heating. The standard conditions were samples heated and cooled at 1.0 °C/min. Other sample conditions were heating slowly at 0.5 °C/min (yet cooling at 1.0 °C/min) and cooling slowly at 0.2 °C/min (after having been heated at 1.0 °C/min). Dynamic measurements were taken at 60-s intervals under a constant strain of 0.015 (value within the linear viscoelastic strain region of the gels under the given conditions) and a 0.1 Hz frequency. Samples were run in triplicate, and a representative sample is presented.

Determination of free thiol groups using Ellman's reagent

The amount of free thiol groups was estimated in samples containing approximately 5 mg of protein per mL dissolved in 0.1 *M* sodium phosphate buffer (pH 8.0) containing 8 M urea. L-Cysteine hydrochloride (8 to 60 mg/L final concentration) was used as a standard. To 50 µL of sample or standard, 250 µL of buffer (0.1 *M* sodium phosphate buffer, pH 8.0, containing 8 *M* urea) was added. To this mixture 20 µL of a 2 mg/mL solution of 5,5 -dithio bis(2-nitrobenzoic acid) in buffer was added and 200 µL of each sample or standard was transferred to a microtiter plate. After 15 min of incubation, the absorbance of the samples and standards was measured at 412 nm using a microtiter plate reader.

Cysteine and methionine determination

Analysis was performed by Ansynth BV (Roosendaal, The Netherlands). Samples underwent oxidation with performic acid for 16 h at 0 °C to 5 °C, followed by acid hydrolysis in 6 *M* HCl for 22 h at 105 °C to 110 °C.

Results and Discussion

To start, the protein isolates were characterized in various ways. First, the total protein content of the isolates was determined, and the results were as follows: Solara 87%, Supra 85%, Classic 84%, Finale 87%, and Espace 86%. Second, the globular protein composition (the percentage of vicilin and legumin in the soluble fraction of the protein sample) was estimated according to the intensity of staining of bands belonging to each protein. The stained samples are shown in Figure 1, and the compositions are summarized in

Table 1. Solara and Espace had the highest (28%) and lowest (21%) legumin contents, respectively, and Finale had the highest percentage of α-subunits (14%). Finally, the minimum gelling concentration of each isolate was determined. For all isolates, the minimum gelling concentration was 16% (w/v). These gels were disrupted by applying a small force (with a glass rod), however, so it was decided to perform all subsequent gelling experiments at 18% (w/v). The 18% (w/v) protein concentration is a higher gelling concentration than needed to make soybean protein isolate gels (Renkema 2001); therefore, it was investigated whether insoluble material was hindering the gel network formation. Samples were spun down, and the supernatant (14% protein by weight) was gelled in the Bohlin rheometer under standard conditions. The strength of the gel was unchanged with respect to the uncentrifuged sample (no results shown); therefore, it was decided to proceed with the unspun sample. It was upon the results of these preliminary tests that it was also decided to use only the soluble fraction for estimating the protein composition of the isolates.

Samples were prepared at pH 7.6, and gelled in a Bohlin rheometer with constant oscillation, taking dynamic measurements throughout the heating and cooling cycles. All the results (Figure 2 and 3) are plotted as storage modulus against temperature. Figure 2b indicates, with arrows and numbers, phase 1 (heating to 98 °C and holding for 30 min) and phase 2 (cooling to 25 °C and holding for 30 min). The plot of phase 1 is only visible for values above 10 Pa. It is for this reason that for some samples the plot of storage modulus is visible only at 98 °C, whereas for others there is a visible increase in storage modulus with temperature during the heating ramp-up to 98 °C. For phase 2, the plots must be read from right to left, following an increasing storage modulus as the temperature decreases.

The 1st comment to make is that when heating/cooling was performed at 1.0 °C/min (standard conditions) or heating at 0.5 °C/min and cooling 1.0 °C/min, there was little difference between the cultivars. Figure 2a through 2e (white circles) show that all the gels formed by heating/cooling at 1.0 °C/min had a final gel strength (at 25 °C) of the same magnitude (about 1000 Pa). Samples heated at 0.5 °C/min showed no divergence from the plot of storage modulus of the standard sample (1.0 °C/min heating/cooling) so no results are shown to avoid confusion. Cultivar differences in gel formation were seen, however, when the samples were cooled slowly at 0.2 °C/min, and for all cultivars there was a difference between the gel formation (at this same rate) in the presence and absence of the thiol-blocking reagent *N*-ethylmaleimide (NEM). Taking 1st cultivar Solara (Figure 2a), this had the highest proportion of legumin (Table 1), and when cooled slowly it had a clear increase in the storage modulus during the initial phase of cooling (98 °C to 80 °C of phase 2 highlighted by a circle). Gel formation within this temperature range in the presence of NEM was different—the plot of gray circles did not have the same increase in storage modulus at the high temperature (see again the circled area in Figure 2a). Because this increased gel formation at high temperatures disappeared in the presence of NEM, it was a strong indication that it was generated by the legumin protein forming disulfide bonds that cooperated within the gel network.

Table 1—Ratios of protein composition of the protein isolate soluble in potassium fosfate buffer, as estimated from the staining intensity of the bands on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[a]

Pea cultivar	Legumin (%)	Vicilin bands ≤ 50 kDa (%)	Vicilin α-subunits (%)
Solara	28	62	10
Supra	25	64	11
Classic	22	67	11
Finale	25	61	14
Espace	20	67	13

[a]The bands belonging to legumin and vicilin are indicated on the SDS-PAGE gel in Figure 1.

Staying with the cultivar Solara, but following the storage modulus at lower temperatures (in phase 2), the development and maturation of the gel network appeared to proceed in the same way in the presence and absence of NEM. This implied that the bonds that strengthened and stabilized the gel networks in this pea cultivar formed independently of any disulfide bonds. The increased number of these bonds (indicated by the increased value of G′) when cooling the gel slowly is believed to be because slow cooling gave more time for the proteins to interact. With more time in motion they were able to orientate themselves into more orderly configurations and maximize their attractive potential (Gossett and others 1984; O'Kane and others 2004c).

For the cultivar Supra (Figure 2b) it can be seen immediately that stronger gels formed only in the presence of NEM. In other words, only when disulfide bonds were absent from the gel network did slow cooling assist a more extensive network interactions to form. From this result, it can be reasoned that in cultivar Supra, the formation of disulfide bonds (black circles in Figure 2b) somehow restricts extensive non–covalent bond formation and strengthening of the gel network that would otherwise happen when cooling slowly. The cultivar Classic (Figure 2c) exhibited the same gelation trends as Supra.

In trying to relate the observations to the protein composition for these 1st 3 cultivars (Solara, Supra, and Classic), it made it seem that the 28% legumin of cultivar Solara (Table 1) should be a critical level of legumin for disulfide bonds not to be detrimental to the gel strength. Maybe below this percentage the network branch points that are believed to be created by disulfide bonding (Gossett and others 1984; Nakamura and others 1984b; O'Kane and others 2004c) were too sparse to bring the network strands close together.

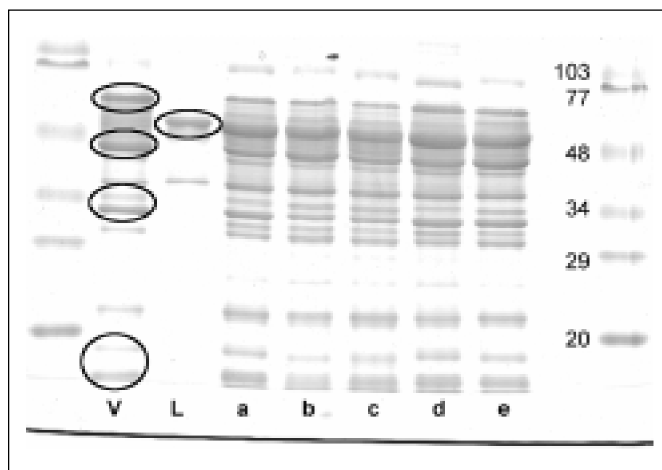

Figure 1—Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of pea protein isolates dissolved in potassium phosphate buffer, run on a 12.5% polyacrylamide Tris-HCl gel. From left to right: V = purified vicilin fraction; L = purified legumin; protein isolates from cultivar (a) Solara, (b) Supra, (c) Classic, (d) Finale, (e) Espace. External lanes are standard markers (molecular weights indicated in kDa on right-hand side). Bands previously assigned to pure proteins are highlighted by circles. The largest band of vicilin (about 70 kDa) was taken separately to calculate the percentage of α-subunits.

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This was therefore tested by adding legumin purified from cultivars Solara and Supra to the protein isolate of cultivar Classic. The level of substitution (40 to 80 mg) boosted the percentage of legumin in the isolate of Classic 28% to 33% (≥ the percentage of legumin in the isolate of cultivar Solara). Gel formation was monitored in the Bohlin rheometer exactly as had been done for the isolates, and the results are presented in Figure 3a and 3b. It was obvious immediately that rather than the quantity of legumin being an issue, it was a cultivar effect. This was concluded because the legumin purified from cultivar Solara added to the Classic isolate sample strengthened the gel system and shifted the shape of the plot toward that of a gel made from 100% isolate cultivar Solara (Figure 3a). The addition of different amounts of legumin purified from cultivar Supra made the plot of storage modulus and temperature equal to that of a gel made from 100% isolate cultivar Supra, which was in fact slightly weaker than that of the gel made from 100% isolate cultivar Classic (Figure 3b).

Having discovered this cultivar specific difference in gelation behavior, an explanation is perhaps to be found in genetically determined differences in legumin, such as the presence of different acidic/basic polypeptides. Under reducing conditions on SDS-PAGE, the size and number of the acidic and basic polypeptides of the purified legumins from cultivars Solara and Supra were the same (no results shown), and therefore this was not investigated further. Instead, the amount of free-sulfydryl groups and the total cysteine content of the purified legumin proteins and the protein isolates were determined. This was done because the isolates of Solara, Supra, and Classic exhibited their differences when disulfide bonds formed within the gel network. The hypothesis put forward at this point was that legumin from cultivar Solara should have less poten-

Table 2—Amount of free-sulfydryl groups and cysteine residues determined to be present in purified legumin of cultivars Solara and Supra, and in the protein isolates of cultivars Solara, Supra, and Classic.

Pea cultivar and protein sample	Free-sulfydryl groups detected (mg/g protein)	Total amount of cysteine residues in the sample (g/kg)
Solara, purified legumin	0.16	6.82
Supra, purified legumin	0.10	7.30
Solara, protein isolate	1.58	7.38
Supra, protein isolate	2.29	7.45
Classic, protein isolate	2.24	8.54

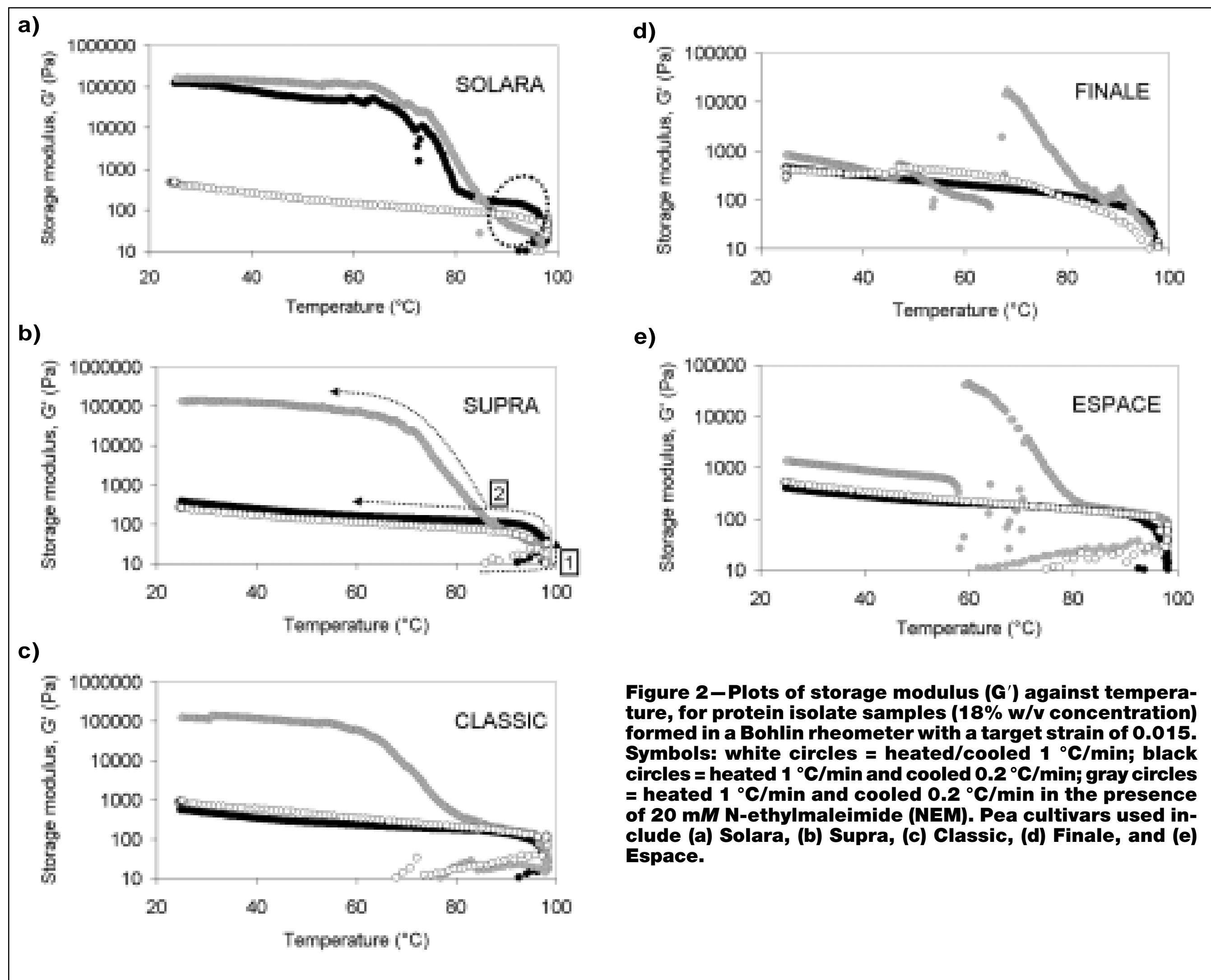

Figure 2—Plots of storage modulus (G′) against temperature, for protein isolate samples (18% w/v concentration) formed in a Bohlin rheometer with a target strain of 0.015. Symbols: white circles = heated/cooled 1 °C/min; black circles = heated 1 °C/min and cooled 0.2 °C/min; gray circles = heated 1 °C/min and cooled 0.2 °C/min in the presence of 20 m*M* N-ethylmaleimide (NEM). Pea cultivars used include (a) Solara, (b) Supra, (c) Classic, (d) Finale, and (e) Espace.

tial for forming disulfide bonds than that from Supra or Classic. Thus, the network branches created by the disulfide bonds in cultivar Solara are so few that they impose very little strength, leaving the aggregate strands free and flexible and able to come close together and form extensive non–covalent bonds during further cooling. Hence, this is why a strong gel could form in both the presence and absence of NEM (Figure 2a). By the same reasoning, the higher number of disulfide bonds in Supra and Classic gel networks could restrict the strand flexibility and prevent an optimal gel network formation with respect to the overall gel strength that could develop. This reasoning seems contrary to the gel-strengthening ability that disulfide bonds are generally considered to have (Utsumi and Kinsella 1985; Shimada and Cheftel 1988; Arntfield and others 1991; Alting and others 2004), but it is not. The amount of legumin present in the pea protein isolates is thus proposed to be too little for a good distribution of disulfide bonds throughout the whole gel network, yet at the same time there is an amount of the disulfide bonds that does not enable their presence to go unnoticed.

Returning now to the analyses performed, the results obtained are presented in Table 2. The purified legumins had a very low free-sulfydryl content. For the protein isolate, the levels were higher than for the purified protein, and the isolate from cultivars Supra and Classic contained slightly more free-sulfydryl groups than the isolate from cultivar Solara. It should be noted that the α-subunits of vicilin are reported to contain 1 (Croy and others 1980; Bown and others 1988; Newbigin and others 1990) or 2 (Casey 2000) cysteine residues per about 70-kDa polypeptide, and so these subunits will have contributed to the figures presented in Table 2 for the isolates for both the free-sulfydryl and total cysteine content. Even accounting for this, the figures in Table 2 indicate the presence of other proteins in the isolate, possibly albumins, that contain significant amounts of cysteine. With respect to the purified proteins, based on an average molecular weight of the legumin of about 58 kDa, the total cysteine contents of the purified proteins correspond to approximately 4 and 5 cysteine residues per molecule for purified legumin from the cultivars Solara and Supra, respectively, which is an intermediary cysteine content relative to those reported by other authors (Croy and others 1980; Casey and Short 1981). Overall, the results presented in Table 2 gave no clear indication that the legumin of Solara has less potential for forming disulfide bonds during heat-induced gelation, and so the hypothesis presented was not proven. The gel strengthening effect of cultivar Solara must therefore be contributed to by other factors. One possibility is that legumin from cultivar Solara contains a large number of hydrophobic residues (that were previously buried in the core of the protein) and that their interaction is what encourages a more extensive gel network formation. A study on the gelation behavior of the glycinin fraction from 4 soybean protein isolates (Riblett and others 2001) showed that the storage modulus of the gels correlated positively with the molar percentage of hydrophobic residues, as well as correlating negatively with the molar percentage of cysteine residues. They therefore concluded that gel-forming properties might be related to more than just protein content—also to the amount and type of amino acid in the fraction. Lack of available protein prevented such a comparison being made of the legumin of Solara and Supra. However, a comment to be added is that if the differing ability to strengthen gels was related to the number of hydrophobic interactions formed in the gel network, the change observed when NEM was added would not have been expected.

Seeking again an involvement of disulfide bond formation, maybe the difference lies in the aggregate size and shape. Reconsidering the effect that NEM was seen to have on the protein isolates from cultivars Supra and Classic (Figure 2b and 2c), it might be speculated that the free-sulfydryl and cysteine residues in these cultivars are spatially very close together when the protein unfolds, and they immediately interact, forming large disulfide-bonded aggregates. Larger aggregates would generate aggregate strands with fewer links from 1 aggregate to the next. With a lesser number of network interactions, ultimately the gel would be weaker (have a lower storage modulus). Adding NEM would remove this driving force to form such large aggregates and enable the benefit of slow cooling to become apparent (as actually observed). In other words, without disulfide-driven aggregation, the unfolded proteins could have remained in motion longer and arranged themselves into more orderly network strands with smaller aggregates and thus a larger total number of bonds contributing to the network strength (as reflected by the increased storage modulus).

Having discussed the impact of legumin on the gelation behavior of the pea protein isolates, now the effect of vicilin's oligomeric composition will be discussed. Figure 2d and 2e show that although the 2 pea cultivars Finale and Espace had the same tendency to form stronger gels when cooled slowly in the presence of NEM, their gel networks were not able to extensively strengthen. Accord-

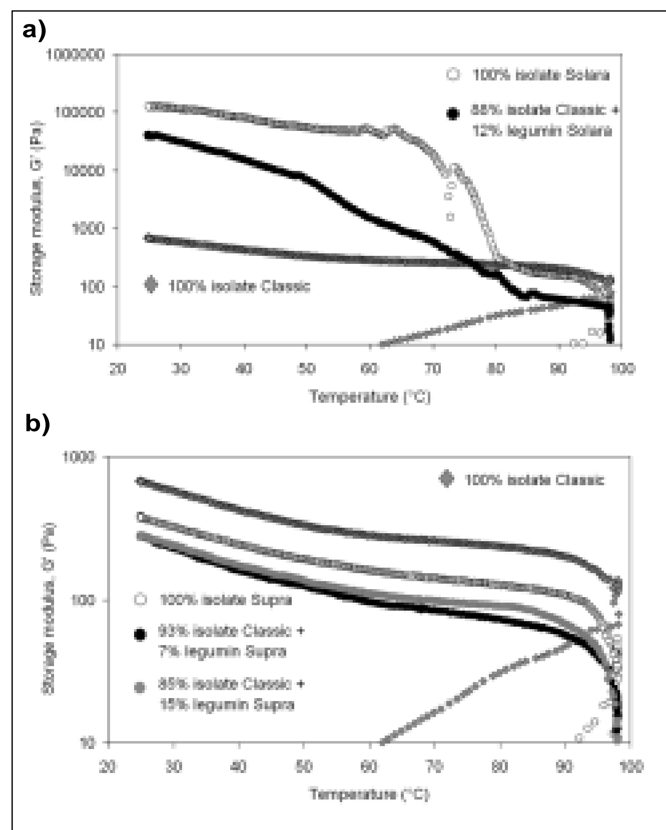

Figure 3—(a) Plot of storage modulus against temperature for 3 different gels, all with 18% (w/v) protein concentration, all heated 1 °C/min and cooled 0.2 °C/min. Symbols: gray diamonds = isolate cv. Classic; white circles = isolate cv. Solara; black circles = isolate cv. Classic with 12% (by weight) substituted by purified legumin protein cv. Solara. (b) Plot of storage modulus against temperature for 4 different gels, 18% (w/v) protein concentration, heated 1 °C/min and cooled 0.2 °C/min. Symbols: gray diamonds = isolate cv. Classic; white circles = isolate cv. Supra; black circles = isolate cv. Classic with 7% (by weight) substituted by purified legumin protein cv. Supra; gray circles = isolate cv. Classic with 15% (by weight) substituted by purified legumin protein cv. Supra.

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ing to the staining intensity of the bands of these 2 cultivars, they were calculated to have an α-subunit content of 14% and 13%, respectively, in Finale and Espace. Although only 3% and 2% more than present in Supra and Classic, it is proposed to be a critical level. It was shown in a previous article (O'Kane and others 2004b) that when a vicilin fraction (50% composed from α-subunits) was added to legumin at a level of 36% (by weight of the total sample), it was able to inhibit gelation at pH 7.6. At this level, the percentage of α-subunits in the sample was approximately 18%. Considering that this was able to prevent the gelation of a purified legumin gel containing disulfide bonds, it seems reasonable that at 14% in the absence of any disulfide bonds, the repulsion of the α-subunits can reduce the gelation ability of the protein isolates Finale and Espace. What was unusual about the gel disruption was that it happened as the temperature dropped. For cultivars Finale and Espace it can therefore be said that the α-subunits override the attractive forces that would otherwise form at lower temperatures during slow cooling. Why the gels strengthens initially is believed to be because there are too few of the repulsive α-subunits to override the strong attraction of the hydrophobic residues. They can override the weaker short-range hydrogen bonds, however. Without the additional strength and stabilization offered by hydrogen bonds, the continuous movement of the repelled strands is believed to disrupt some of the network that has already formed and cause the drop in storage modulus seen in Figure 2d and 2e.

Some studies on globular pea proteins have compared the functionality of crude protein preparations to that of the isolate as a 1st step in determining what should be the ratio of the proteins for an optimum functionality of the protein isolate (Dagorn-Scaviner and others 1986, 1987; Koyoro and Powers 1981). In this article, evidence for relating the gelation behavior of the isolate to its protein composition could be done only for the cultivars Finale and Espace, with respect to the percentage of α-subunits in the vicilin fraction. The contribution of legumin to the pea protein isolate gels was shown to be cultivar-specific and possibly more dependent on spatial proximity of the reactive residues when the protein unfolds than the amount of legumin protein present.

Conclusions

Factors determining the gelation behavior of pea protein isolates include the pea cultivar and the amount of vicilin α-subunits present. More information on the reactivity of the exposed residues is needed to understand the 1st factor.

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