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Analytical Methods

Application of Fourier transform infrared spectroscopy to legume seed flour analysis

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Abstract

The secondary structure of legume (*Phaseolus vulgaris* L. and *Lens culinaris* L.) proteins was investigated by studying the amide I infrared absorption band in whole seed flours, before and after dry heating and autoclaving thermal treatments. The analysis procedure, set up on 7S and different model proteins, shows that the content of β-sheet structures in lentil is higher than in common bean (47% vs. 32%). The dry heating does not appreciably affect secondary structures in lentil, while it causes a reduction of β-sheets (to 13%), an increase of aggregates, and the appearance of random coil structures in common bean. The autoclaving treatment produces high amounts of aggregates in both legumes. However, in lentil, random coil structures are lower than in common bean and some β-sheet structures are still detectable. These results indicate that multimeric heat-induced complexes of legume proteins have a high stability because of the high content in β-sheet structures, in particular in lentil, which may adversely affect protein utilization.

Keywords: Fourier transform infrared spectroscopy; Secondary structure; Heat treatment; Legume proteins

1. Introduction

Legume seeds contains 50–70% storage proteins: 7S (vicilin-like) or 11S (legumin-like) globulins (Derbyshire, Wright, & Boulter, 1976). These proteins are tightly packed into protein bodies of the parenchyma cells until germination, when they are degraded by endogenous proteases. Because of their high nitrogen content, legume seeds represent a major source of dietary protein in the world and the main protein supply in developing countries (FAO/WHO Expert Consultation. Protein quality evaluation. Food & FAO, 1991).

Breeding programmes and protein engineering targets have taken advantage of elucidation of three-dimensional structure of storage proteins to obtain mutants with enhanced nutritional properties (Utsumi, 1992). However, insufficient information is available hitherto on the consequence of processing of legume seeds on protein quality, especially as far as structural changes affecting *in vivo* proteolysis is concerned (Carbonaro, Grant, Cappelloni, & Pusztai, 2000; Deshpande & Damodaran, 1989).

Resistance to denaturation and, probably, to proteolysis has been suggested as a general property of members of the cupin superfamily to which storage globulins belong (Khuri, Bakker, & Dunwell, 2001). This family comprises proteins with different functions sharing a common β-barrel structure that is thought to have originated in a prokaryotic ancestral gene. Each module consists of a β-barrel core domain built from two walls of antiparallel β-sheet associated to a loop domain which predominantly contains α-helices (Ko, Day, & McPherson, 2000). Interest for structural properties of cupins also stems by recognition that a number of them are major plant food allergens, including 7S globulins of soybean, peanut, walnut and lentil, as well as 11S globulins of peanut and soybean (Barre, Borges, & Rougé, 2005). However, relationship between structural properties of globulins and allergenicity has not been clarified yet.

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Despite 7S and 11S storage proteins have been recognized to be evolutionarily related on the basis of their primary structure, they can be distinguished by their oligomeric structure and polypeptide interaction. Indeed, hydrogen bonds and hydrophobic interactions held 7S monomers together in a trimer (M_r 150,000–170,000), whereas disulfide bonds link acidic and basic subunits in pairs in the 11S hexamer (M_r 300,000–450,000) (Adachi et al., 2003; Lawrence, Izard, Beuchat, Blagrove, & Colman, 1994). Both 7S and 11S storage proteins can form high MW aggregates above 75 °C. Although electrophoretic and functional properties of soluble fractions from 7S and 11S legume proteins are similar, we have provided evidence that differences in the association state of these globulins induced by heating may affect their susceptibility to *in vivo* proteolysis (Carbonaro, Grant, & Cappelloni, 2005; Carbonaro et al., 2000). Indeed, it was found that while native legume globulins were well digested (92–95%), after thermal denaturation protein digestibility of 7S globulin was reduced to 88%, while that of 11S globulin to 79% only (Carbonaro et al., 2005).

Structural aspects that may account for the stability of legume proteins upon heat-processing and/or gastro-intestinal digestion, such as in particular the secondary structure, have not been thoroughly investigated (Carbonaro et al., 2000; Deshpande & Damodaran, 1989). Unfortunately, structural analysis of plant oligomeric proteins and of conformational changes that occur during processing is hampered by their low solubility. Therefore, the study of such proteins needs techniques, such as solid-state fluorescence, that do not require to bring the system in a fully soluble state, that would result in significant alteration of the protein structure (Bonomi, Mora, Pagani, & Iametti, 2004).

Fourier transform infrared (FT-IR) spectroscopy, a vibrational spectroscopic technique, can be a powerful tool to get information on the secondary structure of proteins by studying in detail the different contributions to the amide I band, which arises from the stretching vibrations of C=O in the peptide bonds (Jackson & Mantsch, 1995). The vibration energies of the carboxyl group depend indeed on the different conformations of the protein, such as β-sheet and α-helix structures, β- and α-turns, and inter- or intra-molecular aggregates. The determination and the assignment of the spectral components of the amide I band can then provide information on the protein secondary structure. Additionally, the relative intensity of a spectral component associated to a given structural conformation is related to the relative content of the conformation itself. Several FT-IR studies on the conformation of various food proteins, including plant globulins, are reported in the literature (Choi & Ma, 2005; Meng & Ma, 2001). Moreover, a detailed FT-IR analysis of the components of the amide I band allows to monitor the conformational changes of food proteins that occur upon processing (Murayama & Tomida, 2004).

In the present study, the secondary structure of proteins in whole seed flours of two legumes, common bean (*Phaseolus vulgaris* L.) and lentil (*Lens culinaris* L.) is investigated by FT-IR spectroscopy to elucidate how common bean and lentil proteins behave upon dry and wet thermal treatments and to get some insights on the unfolding process that occurs upon thermal processing. FT-IR measurements were performed on powdered samples embedded in KBr pellets, which is convenient in the case of proteins with limited solubility, such as heat-treated legume proteins. The amide I band of model proteins, such as 7S globulin purified from common bean seed and commercially available proteins, was first analyzed by using both the Fourier self-deconvolution (FSD) procedure and curve fitting analysis. For common bean, the amide I band of the raw and heat-treated samples (both autoclaved and dry thermally treated) was studied by using the same analysis procedure. Finally, the amide I band of lentil, a legume containing the 11S protein, was studied in both the native and denatured states, to be compared with that of common bean (containing the 7S protein).

The present FT-IR study provides information on the average structure of all the proteins in the investigated flours, and on their behaviour in the food matrix upon dry and wet thermal treatments. Since obtained results can be of interest for the important topic of protein bioavailability, the relationship between structural and nutritional properties of the investigated legume proteins is finally addressed.

2. Materials and methods

2.1. Samples

High-purity 7S globulin was prepared from common bean (*Phaseolus vulgaris* L.) according to Carbonaro et al. (2005) and freeze-dried. Concanavalin A, α-lactalbumin, β-lactoglobulin and α-casein were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). All proteins were stored at −30 °C until use.

Dry seeds of common bean (*P. vulgaris* L.) and lentil (*L. culinaris* L.) were obtained from the local market. Raw powdered samples were obtained in a Cyclotec 1093 Tecator. Sample seeds, after soaking in water for 2 h (1:4, w/v), were autoclaved with the soaking water for 20 min at 120 °C (1 atm) and then freeze-dried and powdered. Thermally treated samples were instead prepared in a thermostatic oven at 120 °C for 30 min and for 20 h.

Protein content (N × 6.25) was determined by the Kjeldahl method (AOAC., 1999). Protein content, on a dry basis, was 95% for 7S globulin, 27% for common bean and 25% for lentil.

2.2. FT-IR measurements

FT-IR measurements were performed on pellets of sample powder mixed with KBr. The sample quantity (between 2 and 6 mg) in KBr (200 mg) was chosen in order to optimise the pellet transmittance and to obtain a well detect-

able absorption in the spectral region of the amide I and amide II bands. Measurements were performed in the mid-infrared region, with a resolution of 4 cm^{-1}, by using a Bruker IFS66 V interferometer (Bruker Optics, Ettlingen, Germany) working under vacuum to avoid intense spectral components due to atmospheric CO_2 and H_2O. By measuring the infrared intensities transmitted by a pure KBr pellet (I_0) and that transmitted by the pellet containing the sample (I_S), the optical density $O_d(\omega) = \ln[I_0(\omega)/I_S(\omega)]$ was obtained, which is proportional to the absorbance of the sample. Through measurements carried out on pellets containing different sample quantities, it was verified that the absorption spectral shapes were well reproducible, while the absorption intensities were proportional to the sample quantity.

Data analysis was mainly performed by using both curve fitting procedures and the (FSD) method of OPUS software (Bruker Optics).

3. Results

3.1. Model proteins

In order to get coherent information on the components of the amide I band in different systems, all the measured $O_d(\omega)$ spectra were analyzed with the same procedure. Details of the analysis procedure are reported below for 7S globulin storage protein.

Fig. 1 shows the $O_d(\omega)$ spectrum of the 7S globulin in the spectral region 1000–1750 cm^{-1}. The main spectral features consisted of four intense bands, centered around 1100, 1420, 1520 and 1660 cm^{-1}. The intense absorption band around 1100 cm^{-1} (band A in Fig. 1) can be attributed to C–O and C–C stretching, and also to CCH, COH and HCO deformational vibrations (Naumann, 2001). The band around 1420 cm^{-1} (band B) falls in the region of C–H bending modes, and is most likely originated from the deformational vibrations of the CH_2 functional groups (Bhattacharjee et al., 2005). Finally, the bands around 1520 and 1660 cm^{-1} are assigned to N–H bending (amide II) and C=O stretching (amide I), respectively. The low-intensity band detected around 1240 cm^{-1}, can be ascribed to the oppositely phased N–H bending mode (amide III) (Naumann, 2001).

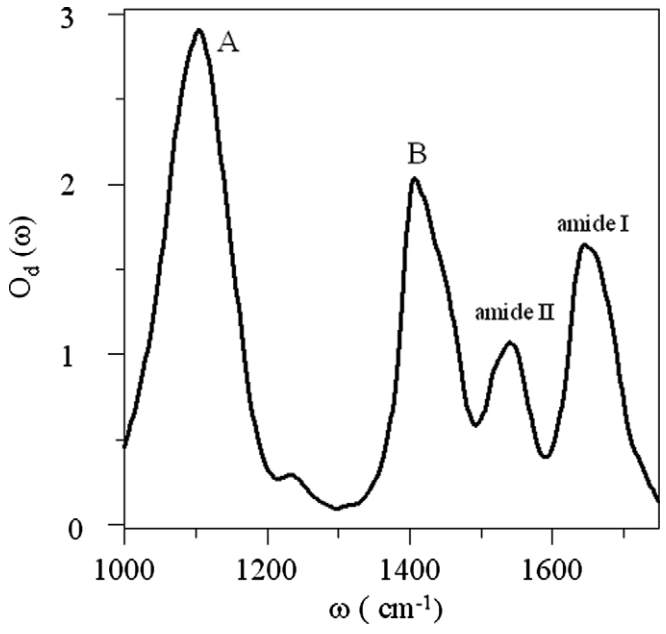

Fig. 1. The optical density $O_d(\omega)$ of the 7S globulin. The sample amount in the KBr pellet was 1% in weight.

A least square fit with Gaussian profiles of the $O_d(\omega)$ spectrum in the 1300–1800 cm^{-1} range first allowed to isolate the amide I band. A FSD procedure (with bandwidth = 5 cm^{-1}, resolution enhancement factor = 2) was then applied to this band in order to better resolve its components. A multi-component fitting of the deconvoluted band finally allowed to identify its components and, in particular, to determine the corresponding peak frequencies (ω_i). It is worth noting that, in all the spectra considered in the present work, the maximum number N of components which can be safely identified in the deconvoluted amide I band does not exceed $N = 5$. Indeed, higher N values do improve the fit quality, but the strong correlation between best fit parameters prevents any definitive assessment on peak frequencies and intensities of the different components.

Infrared analysis of the amide I band is aimed not only at identifying its components through the corresponding peak frequencies, but also at determining the corresponding relative intensities, which are diagnostic of the protein secondary structure. However, it was verified that the integrated intensities I_i, as obtained from the fit of the deconvoluted amide I band, can be significantly affected by the FSD procedure itself. Therefore, a multi-component fit of the $O_d(\omega)$ spectrum in the 1300–1800 cm^{-1} range was performed by using Gaussian components. In the frequency region of the amide I band, $N \leqslant 5$ components were employed, centered at the ω_i values previously determined.

The employed procedure provides reliable values of the peak frequency ω_i and of the relative spectral weight $W_i = I_i / \sum I_i$ of each i-contribution to the amide I band. Fig. 2 shows the resulting best fit profile of the measured $O_d(\omega)$ spectrum in the frequency region of the amide I band in the 7S globulin case. In Fig. 2, the Gaussian components of the amide I band are shown separately (full lines), and are superimposed to the tails of components (dashed lines) centered at lower frequencies, mainly due to the amide II band, and higher frequencies. It is worth noting that the employed analysis procedure allows a reliable identification of the amide I components, even in the case of significant overlap with spectral contributions at lower and higher frequencies.

The ω_i and W_i values obtained in the 7S globulin case are reported in Table 1. The component at 1638 cm^{-1} is assigned to β-sheet modes (henceforth identified as β component), the one at 1654 cm^{-1} to α-helix modes (α component)

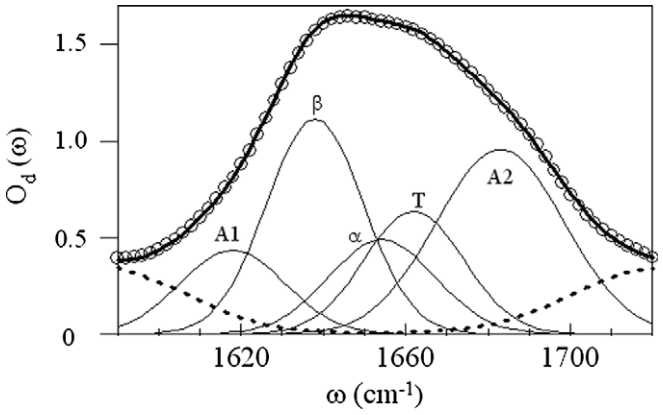

Fig. 2. The optical density $O_d(\omega)$ of the 7S globulin in the amide I spectral region (open circles) reported with its best fit (full marked line). The Gaussian components of the amide I band (full lines) and the tails of high and low-frequency contributions (dashed lines) are also shown.

(Bhattacharjee et al., 2005; Meng & Ma, 2001). The 1618 cm^{-1} component is assigned to inter-molecular aggregates (A1 component), the 1683 cm^{-1} one to intra-molecular aggregates (A2 component) (Bandekar, 1992; Ismail, Mantch, & Wong, 1992; Lefevre & Subirade, 2000), and the 1666 cm^{-1} component can be ascribed to vibration modes originated by turns in the β-sheet structures (T component) (Bhattacharjee et al., 2005; Meng & Ma, 2001).

The procedure applied to 7S globulin was then used in the analysis of the $O_d(\omega)$ spectra of the model proteins selected in the present work: concanavalin A, α-lactalbumin, β-lactoglobulin, α-casein (see Section 2). The resulting values of peak frequencies ω_i and relative spectral weights W_i are reported in Table 1.

Results reported in Table 1 show that the 7S protein is characterized by a high amount of β-sheet (β, 28%) and of aggregate (A1 and A2, 43%) components. The amount of β-sheet structure in concanavalin A (β, 36%) was higher than in 7S globulin, while aggregate components have comparable weights. In α-lactalbumin, the dominating component is given by α-helix structures (α, 42%), while in β-lactoglobulin the main components are due to β-sheet (β, 37%) and intra-molecular aggregate (A2, 32%) structures (Kabsch & Sander, 1983). Finally, in α-casein the dominant contribution (62%) is centered at 1645 cm^{-1}. This component, not detectable in the previous cases, can be assigned to random coil structures (RC component) (Sawyer & Holt, 1993).

As to above assignment, we notice that the peak frequencies of the α, β and turn components slightly vary in different proteins since the corresponding structures are very sensitive to the specific microenvironment (Murayama & Tomida, 2004). As to the comparison with literature data, it is worth noting that the peak frequencies of a given protein may be slightly affected by the measurement conditions and analysis procedure. Especially for aggregates, the assignments reported in Table 1 might be to some extent controversial. Indeed, different analyses reported in the literature are often based on a number N of components in the amide I band higher than 5, which can provide results different from those reported in the Table. For example, the intense component associated to intra-molecular aggregates (A2 component) probably includes the high-frequency vibrational modes reported for the carboxyl group (Meng & Ma, 2001; Yu, 2005). However, as noted above, best fit parameters are strongly correlated when $N > 5$, which prevents, in particular, a reliable evaluation of the relative spectral weights W_i.

Results obtained for model proteins are in agreement with structural data coming from the X-ray crystallographic studies. Such studies indicate that 7S and concanavalin A are characterized by a high content of β-sheet and a low content of α-helical structures (Ko et al., 2000; Lawrence et al., 1994). The same characteristics have been also observed in β-lactoglobulin (Brownlow et al., 1997), whereas the opposite has been found for α-lactalbumin

Table 1
Assignment, peak frequencies ω_i (cm^{-1}) and relative spectral weights $W_i = I_i / \sum I_i$ of 7S globulin and model proteins

		7S	Concanavalin	α-Lactalbumin	β-Lactoglobulin	α-Casein
A1	ω_{A1}	1618	1612	1617	1617	1613
	W_{A1}	0.12	0.09	0.11	0.10	0.08
β	ω_{β}	1638	1633	1634	1636	
	W_{β}	0.28	0.36	0.09	0.37	ND[a]
RC	ω_{RC}					1645
	W_{RC}	ND	ND	ND	ND	0.62
α	ω_{α}	1654	1652	1652	1660	
	W_{α}	0.13	0.11	0.42	0.13	ND
T	ω_{T}	1666	1666	1672	1672	
	W_{T}	0.16	0.16	0.14	0.08	ND
A2	ω_{A2}	1683	1683	1690	1690	1686
	W_{A2}	0.31	0.28	0.24	0.32	0.30

The error on ω_i values is ±2 cm^{-1} and that on W_i values is ±0.02. Symbols in the first column refer to inter-molecular aggregate (A1), β-sheet (β), random coil (RC), α-helix (α), β-turn (T) and intra-molecular aggregate (A2).

[a] ND = not detectable.

(Chrysina, Brew, & Acharya, 2000). Finally, several spectroscopic techniques have revealed that α-casein, an uncommon unstructured protein, contains at least two-third random coil conformation in the native state (Sawyer & Holt, 1993 and refs. therein).

3.2. Common bean and lentil

The analysis procedure employed for 7S globulin and model proteins was applied to common bean and lentil samples, in order to determine the spectral contributions to the amide I band.

Typical $O_d(\omega)$ spectra measured in the spectral range of the amide I band for common bean samples: raw (RB), dry thermally treated (TB), and autoclaved (AB) samples (see Section 2) are reported in Fig. 3. In each panel, the different components and resulting best fit profile are shown separately. Since the spectra of Fig. 3 were measured with the same sample amount in the pellet, it is evident that the thermal treatment reduces the intensity of the amide I band, but does not drastically change its spectral shape. On the contrary, the autoclaving treatment, besides strongly reducing the amide I band intensity, drastically changes its spectral shape, which is broader and less structured than in the raw and dry thermally treated common bean samples. The peak frequencies and relative spectral weights of the components of the different common bean samples are reported in Table 2a.

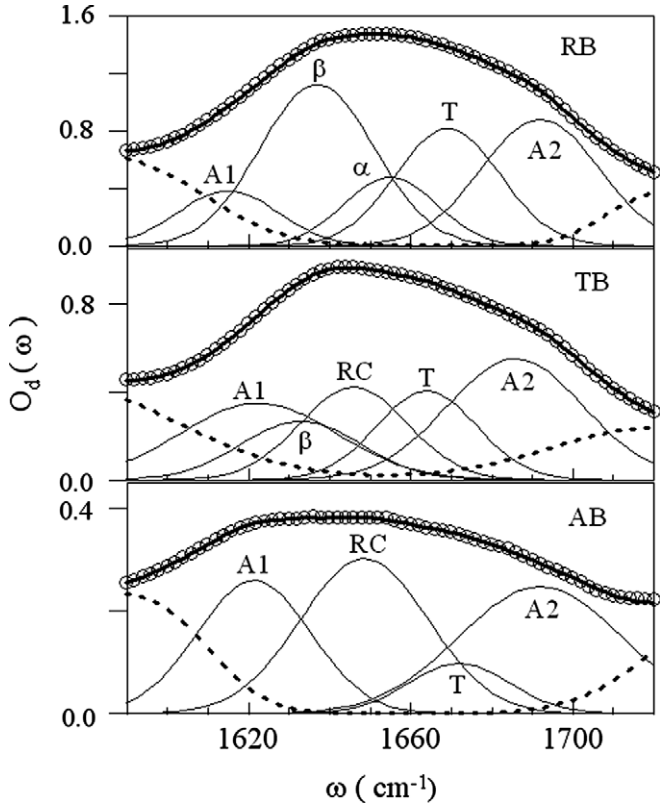

Fig. 3. The optical density $O_d(\omega)$ of bean samples in the amide I spectral region (open circles). RB: raw common bean; TB: dry thermally treated common bean; AB: autoclaved common bean. The protein quantity in the KBr pellet was 1% in weight for all samples. The full marked lines are the best fit to data. The Gaussian components and the tails of high and low-frequency contributions are reported in full and dashed lines, respectively.

For the raw common bean sample, the amide I band closely resembles that of 7S globulin, since the most significant contributions are the β-sheet (β) and molecular aggregate (A1 and A2) components (see Table 2a). However, no negligible contributions to the spectral weight are due to α-helix (α) and β-turn (T) components. As far as the dry thermally treated samples is concerned, it is worth noting that the spectra measured on samples heated for 30 min and 24 h are not appreciably different. For either sample, a complete disappearance of the α-helix (α) contribution was observed. Moreover, the β-sheet contribution (β) strongly decreases (from 32% to 13%) and slightly shifts to lower frequencies. This effect, already observed in red bean (Meng & Ma, 2001) and in buckwheat (Choi & Ma, 2005) globulins, has been attributed to protein unfolding and denaturation. On the other hand, a good description of the amide I band of the dry thermally treated sample requires an important contribution (18%) peaked around 1646 cm^{-1}, to be ascribed to random coil (RC) structures. In the autoclaved common bean sample, the dominant contributions to the amide I band are due to random coil (RC, 33%) and aggregate (A1 + A2, 59%) contributions, while β-turns (T) give a small but not negligible contribution.

The $O_d(\omega)$ spectra of raw lentil (RL), dry thermally treated lentil (TL) and autoclaved lentil (AL) samples in the spectral range of the amide I band are reported in Fig. 4. As in the common bean case, different extents of dry thermal treatment (30 min and 24 h) do not produce appreciable differences. Both thermal and autoclaving treatments

Table 2
Assignment, peak frequencies ω_i (cm^{-1}) and relative spectral weights $W_i = I_i / \sum I_i$ of (a) raw common bean (RB), dry thermally treated common bean (TB) and autoclaved common bean (AB); (b) raw lentil (RL), dry thermally treated lentil (TL) and autoclaved lentil (AL)

		(a) Common bean			(b) Lentil		
		RB	TB	AB	RL	TL	AL
A1	ω_{A1}	1615	1622	1621			1617
	W_{A1}	0.10	0.22	0.25	ND[a]	ND	0.19
β	ω_{β}	1637	1633		1638	1638	1629
	W_{β}	0.32	0.13	ND[a]	0.47	0.46	0.07
RC	ω_{RC}		1646	1649			1649
	W_{RC}	ND	0.18	0.33	ND	ND	0.23
α	ω_{α}	1655			1657	1657	
	W_{α}	0.11	ND	ND	0.08	0.08	ND
T	ω_{T}	1669	1664	1672	1672	1672	1669
	W_{T}	0.21	0.16	0.08	0.22	0.17	0.11
A2	ω_{A2}	1692	1685	1692	1693	1694	1690
	W_{A2}	0.26	0.31	0.34	0.23	0.29	0.40

The error on ω_i values is ±2 cm^{-1} and that on W_i values is ±0.02. Symbols in the first column refer to inter-molecular aggregate (A1), β-sheet (β), random coil (RC), α-helix (α), β-turn (T) and intra-molecular aggregate (A2).

[a] ND = not detectable.

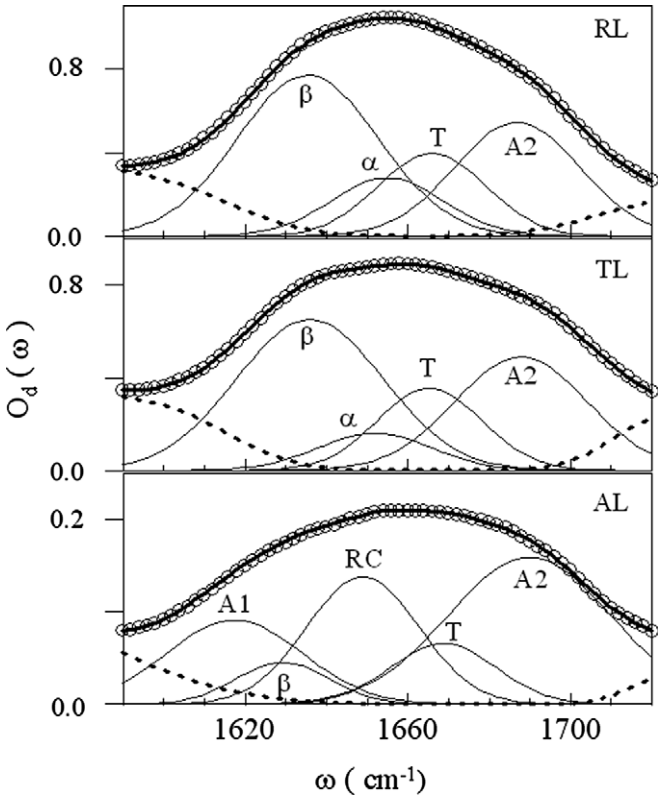

Fig. 4. The optical density $O_d(\omega)$ of lentil samples in the amide I spectral region (circles). RL: raw lentil; TL: dry thermally treated lentil; AL: autoclaved lentil. The protein quantity in the KBr pellet was 1% in weight for all samples. The full marked lines are the best fit to data. The Gaussian components and the tails of high and low-frequency contributions are reported in full and dashed lines, respectively.

significantly reduces the intensity of the band. Differently from the common bean case, the band spectral shape does not drastically change in the autoclaved sample. The peak frequencies and relative weights of the spectral components observed in raw, dry thermally treated and autoclaved lentil samples are reported in Table 2b. Differently from the raw common bean sample, we verified that the amide I band of the raw lentil sample can be well described by only four contributions: the β-sheet component is dominant, while the inter-molecular contribution is not detectable. The spectral weights of the four components are not appreciably affected by the dry thermal treatment. In the autoclaved sample, the α contribution becomes not detectable and the β contribution is strongly reduced (from 47% to 7%) and remarkably shifts to a lower frequency. It is worth noting that this shift, differently from the common bean case, only occurs under a severe thermal treatment. In addition, the A1 and RC contributions become important and the A2 contribution significantly increases (from 23% to 40%).

4. Discussion and conclusions

In the present work, the secondary structure of different proteins is investigated by studying their FT-IR absorption spectrum. In particular, the amide I bands measured in model proteins, such as 7S globulin and commercially available proteins, and then in common bean and lentil, both dry thermally treated and autoclaved, were analyzed. The employed procedure, which efficiency is proved by the results obtained for model proteins, provides a self-consistent scheme which allows to safely compare the relative spectral weights of the amide I band components in different samples. Detailed information on the alterations in the secondary structure of legume proteins in whole seed flour, due to a dry thermal treatment and to a more severe thermal treatment, such as autoclaving, has thus been obtained.

Present results first show that proteins in untreated common bean and lentil flours have a high content in β-sheet structures. This is in agreement with previous findings showing that proteins of the cupin family, that includes storage globulins from monocotyledonous and dicotyledonous seeds, contain a high level of β-sheet structure (Marcone, Kakuda, & Yada, 1998).

A comparison between common bean and lentil proteins, that are dominated by 7S and 11S globulins, respectively, indicates that the spectral component due to β-sheet structures in lentil is higher than in common bean (47% vs. 32%). After the dry thermal treatment, these structures are completely conserved in the case of lentil, while they are considerably reduced (to 13%) in common bean, thus suggesting a different pathway towards extensive aggregation. Indeed, after dry heating, an increase of both low- and high-frequency bands, associated to inter- and intra-molecular antiparallel β-sheet aggregates, and the appearance of random coil structures is observed only in the common bean case. It is also worth noting that α-helix structures are not appreciably affected by dry heating in lentil, while they are reduced to an undetectable quantity in common bean. Therefore, unfolding and structural reorganization upon dry heating occur only for protein in common bean flour, while a slight destabilization of the protein structure takes place in the case of lentil proteins.

The autoclaving treatment causes loss of secondary structure and increase in the A1 and A2 aggregates of proteins in lentil flour (see Table 2b). It is worth recalling that an increase of antiparallel β-sheet aggregates upon thermal processing has been reported for soy and buckwheat proteins (Choi & Ma, 2005; Prudêncio-Ferreira & Arêas, 1993). Although autoclaving produces a similar amount of aggregated complexes (60%) in both legumes, the fraction of random coil structures observed in autoclaved lentil is lower than in common bean proteins (23% and 33%, respectively). Moreover, the β-sheet contribution, which is negligible in autoclaved common bean, is strongly reduced but still detectable in autoclaved lentil. These results clearly confirm a higher stability of lentil than common bean proteins. A very high content in β-sheet structures (47%) in lentil and stabilization of native structure of the 11S hexamer by disulfide bonds, linking acidic and basic subunits, are likely to account for the different behaviour.

On the above basis, we provide evidence that multimeric heat-induced complexes of proteins in common bean (*P. vul-*

garis) and lentil (*L. culinaris*) flour have a high content of antiparallel β-sheet structures. In fact, random coil conformation, typical of naturally unstructured casein proteins, was detected only in low amounts in dry thermally treated legume samples. This may suggest that aggregation of β -sheet structures occurred in early steps of protein denaturation.

As far as the relationship between structural and nutritional properties is concerned, we recall that a high level of β-sheet structure has been hypothesized to be responsible for low access to proteolytic enzymes and might, therefore, decrease the protein digestibility (Yu, 2005). However, low susceptibility to proteolysis of native globulins has recently been questioned by the results of *in vivo* ileal digestibility studies (Carbonaro et al., 2000; Carbonaro et al., 2005; Crevieu et al., 1997). On the other hand, adverse effect of heat treatment on digestibility of legume proteins has been reported (Carbonaro, Cappelloni, Nicoli, Lucarini, & Carnovale, 1997; Carbonaro et al., 2005; Deshpande & Damodaran, 1989). In particular, previous studies on the effect of thermal denaturation on intestinal digestibility of purified 7S and 11S globulins showed that autoclaving reduced digestibility of both proteins. We observe that the amount of β-sheet structures and the thermal stability of lentil (containing 11S globulins) are higher than those of common bean (containing 7S globulins). This result appears to be consistent with the intestinal digestibility which, after thermal treatment, resulted to be lower for 11S than for 7S proteins (79% and 88% digestibility, respectively) (Carbonaro et al., 2005).

Unknown properties of highly stable β complexes may be responsible, at least in part, for adverse effects, other than amino acid imbalance, that have been observed after consumption of legume globulins (Rubio, Grant, Caballé, Martinez-Aragon, & Pusztai, 1994). These may include preserved antigenicity, as it has been described for storage proteins from peanut and other structurally related major food allergens of the cupin family (Barre et al., 2005). Structural basis of the resistance of β-lactoglobulin, a well known antigenic protein, to peptic and chymotryptic digestion has previously been suggested to be related to a high content in β-sheet structure and disulfides (Reddy, Kella, & Kinsella, 1988).

In conclusion, although structural aspects that may account for resistance to gastro-intestinal digestion of plant proteins need to be investigated further, our results can be a significant support for a further understanding of the bioavailability of legume proteins, an important problem which requires the join efforts of a number of experimental techniques. Furthermore, information from the present study could be useful in improving utilization of legumes as functional ingredients.

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