Abstract An investigation was conducted on the thermal behaviour of meals of six lupin species (*L. albus*, *L. angustifolius*, *L. mutabilis*, *L. luteus*, *L. pilosus* and *L. hispanicus*) by monitoring transition temperature, transition enthalpies and activation energy by differential scanning calorimetry (DSC). Results showed two thermal transitions in the meals of all the species at about 27 °C and 53 °C for the first and second endotherms, respectively. The thermal parameters can be used as indexes of homology. *L. pilosus* proteins showed to be strongly resistant to transition and in opposition, *L. luteus*, *L. hispanicus* and *L. mutabilis* proteins presented the lower transition values. When the transition parameters are also considered, it can be concluded that cultivated species are quite different from the wild species in that they are more resistant to thermal transition.

Keywords DSC · Thermal behaviour · *Lupinus* · Meals

Introduction

Lupins have been used intensively since ancient times both in agricultural practices and as food and feed. Some of the lupin species have been used for more than 3000 years, as in the case of the use of lupin in Egypt. In general, this genus presents high protein levels. Besides, the amount of other macromolecules is generally very low. For instance, in *L. luteus*, the amount of starch is only 1–2% [1]. So, the use of lupin species as a protein source may be envisaged. The knowledge of thermal properties is an important aspect to be considered as they are related to the functional properties. Processing by extrusion-cooking of protein-rich materials is a challenging technology and is one of the cases in which knowledge of the transition pattern is an important aspect.

Legume seeds contain a variety of proteins playing structural, metabolic or storage roles. The storage proteins are the major proportion of the total protein amount. It is generally accepted that there are a limited number of storage proteins, historically referred to as legumin and vicilin (or in another classification as 11S and 7S) [2].

Since differential scanning calorimetry (DSC) has proved to be a valuable tool in the analysis, investigation and fingerprinting of a wide array of proteins and proteinaceous systems, it has been applied to the study of the composition of a number of seed meals. For a particular protein under specified conditions, DSC measures a number of parameters that are characteristic of that protein and so helps to clarify the question of homology among seed storage proteins. These include temperatures of thermal transition, normally recorded as the temperature of maximum transition rate (T\text{max}), or the extrapolated temperature of the onset of transition (T\text{m}), and the heat change associated with the process.

DSC has already been applied to the investigation of legume protein systems [2]. The work of Wright and Boultier [2] on the homology between lupin and other plant seed globulins is the pioneer calorimetric study on lupin proteins. Nevertheless, bibliography concerning lupins is quite scarce [2, 3]. Sousa et al. [3] studied the thermal stability of 7S and 11S globulin fractions extracted from lupin seed (*L. luteus*) flour. On the contrary, the soy globulins have been extensively studied. There have already been reports on the thermal properties of the soy protein isolates or of its fractions [4, 5, 6] and stability as a function of water content [7, 8, 9]. Also, the effect of pH and of different salts on the stability of the soy globulins using DSC has been described [10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. Kitabatake et al. [7] related the transition behaviour, as measured by DSC, to the extrusion texturisation of soy isolates. Other studies report the thermal analysis of different vegetable protein sources like chickpea [18] and oat globulin [19]. This study of...
the DSC investigations on thermal properties of six different lupin flours aims to test two general goals: the applicability of DSC procedure to characterise lupin species and try to establish possible homology between them.

**Materials and methods**

**Materials**

Six different Lupinus species seeds were used in this study. L. albus, L. angustifolius, L. mutabilis and L. luteus are cultivated species and L. pilosus and L. hispanicus are wild species. Samples were obtained from a national germplasm collection.

**Methods**

**Milling**

Seed samples were ground in a Falling number 3100 mill to pass a 0.8 mm sieve.

**Thermal analysis by DSC**

For DSC analysis a Shimadzu DSC 50 equipped with a TA 50 SI thermal analyser was used. Helium (99.95% purity) was the purge gas and flowed at approximately 20 mL min$^{-1}$. The calorimeter was calibrated according to standard procedure established in the manufacturer’s user manual, using indium (n.m. 156.6 °C, Hf=28.45 J g$^{-1}$) and deionised water (m.p. 0 °C, Hm=333 J g$^{-1}$).

Mills were equilibrated before testing at aw=0.05 in closed vessel containing a saturated NaOH solution. Samples of ca. 10 mg were weighed into aluminium pans (inner volume: ca. 30 mL) to the nearest 0.1 mg, and covers were hermetically sealed into place. An empty, hermetically sealed aluminium pan was used as reference. Prior to analysis of samples, the baseline was obtained with an empty, hermetically sealed aluminium pan. Meals were subjected to the following programme: heating at a rate of 1 °C min$^{-1}$ from 22 °C to 100 °C. Thermal transition was defined in terms of onset temperature (expressed as $T_{on}$) and peak or transition temperature ($T_d$). The manufacturer’s software programme was used to analyse and plot the thermal data. An effort was made to have all samples of the same order of weight but they were not exactly the same; therefore, the DSC scans reflects this small sample variation. Heat of transition or enthalpy H (J g$^{-1}$) was evaluated from peak areas and results expressed per weight (g) of protein, using as reference the values of nitrogen evaluated by Kjeldhal method (Table 1).

Due to the low amount of available samples, nitrogen content results are only the average of three replicates. The transition kinetics of lupin meals was studied by DSC, using the heat evolution method of Borchardt and Daniels as reported by Danielenko et al. [13]. All results are at least the mean values of four replicates.

**Results and discussion**

DSC analysis of lupin meals

In the thermograms of all lupin meals, two distinguishable transitions were observed, but the different species showed different patterned endotherms.

The observed differences between the thermograms may be explained by different factors. One of them is the proportion of the different protein fraction in each species. In this study we used different taxonomic samples containing different concentrations of the different components, and so it may be reflected in the magnitude of the endotherms [2]. Another aspect to be considered is the observed differences in the transition temperatures and in the related enthalpies profiles (Fig. 1). These properties are not linked to the protein amount (as results are expressed per g of protein) but to the protein properties that seem to be different from species to species. Analysing the results for temperature and enthalpy of the first and second endothermic transitions, distinguishable groups can be formed between the studied species for both. The average temperatures of the endotherms are not significantly different for the majority of the species, except for L. mutabilis and L. luteus which present higher transition temperatures. Regarding the related enthalpies, L. pilosus and L. angustifolius needed more energy for transition. Nevertheless, those species showed the values of temperature for onset of transition in the lower range.

As lupin meals are mainly composed of protein it is reasonable to assume that the detected transitions are related to denaturation of protein fraction, eventually fractions 7S and 11S as referred to in bibliography. So it may be assumed that L.uteus and L. mutabilis proteins need higher temperatures to onset denaturation, but, once the process starts, the energy necessary to accomplish denaturation is lower than that needed for the other species. In contrast, the specie that seems to be more resistant to heat denaturation is L. pilosus.

**Transition kinetics of lupin meals**

The transitions of proteins are a cooperative phenomenon that is accompanied by a significant increase of heat, seen as an endothermic peak in the DSC thermogram [20].

The extrapolated temperature of the onset of transition ($T_{on}$) and the transition temperature ($T_d$) indicate the thermal stability of the proteins. These temperatures can be changed by the heating rate and by the protein concentration. The cooperativity of protein unfolding has

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**Table 1** Protein (N×6.25) content of lupin species

<table>
<thead>
<tr>
<th>Lupinus species</th>
<th>g protein/100 g D.M.</th>
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</thead>
<tbody>
<tr>
<td>L. albus</td>
<td>39.0</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>35.7</td>
</tr>
<tr>
<td>L. mutabilis</td>
<td>43.0</td>
</tr>
<tr>
<td>L. pilosus</td>
<td>25.7</td>
</tr>
<tr>
<td>L. luteus</td>
<td>39.4</td>
</tr>
<tr>
<td>L. hispanicus</td>
<td>41.7</td>
</tr>
</tbody>
</table>