MODERN APPROACHES AND RECENT ACHIEVEMENTS IN STUDYING THE IMPACT OF WHITE LUPIN SEED PROTEINS ON HUMAN NUTRITION AND HEALTH

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Lupin seeds, as other legume seeds, are increasingly being shown to contain a number of bioactive molecules and functional compounds which concur to the remarkable properties of this still underexploited seed from both human health and techno/functional viewpoints.

In this respect, the examples of industrial foods based on including lupin flours or specific components thereof are growing, giving rise to a number of specialities and consolidated enriched food products, including pasta, bakery and dairy products.

Meanwhile, various pharmaceutical and nutraceutical companies consider some of the lupin seed components as strategic molecules for the prevention, and even therapy, of various pathologies, including those dismetabolic syndromes which are typical of the affluent countries, the so-called 'civilisation diseases' (Duranti, 2006). In addition, synergies, as well as potential antagonisms, among various functional components are likely to occur and are worth to be considered first from the scientific viewpoint and secondly as further potentialities or limitations.

The mentioned areas of technological and nutri/functional exploitation are quickly expanding, based on the tight links between basic and applied research, which our group is contributing to with a number of experimental approaches, scientific and technical activities summarised in the term 'molecular nutraceutics' (Scarafoni *et al.* 2007). Specifically, our group is focussing on the characterisation of lupin (and other legume seeds) protein molecular properties with the goal of identifying biological activities which may be relevant to the best exploitation of these major components. Because of the variability of lupin protein components, the multifactorial nature of the phenomena involved and the complexity of seed/food matrices, our approach consists in the isolation of specific seed proteins with modern and innovative molecular tools, their fine structural/molecular characterisation and the assessment of the biological effects of the highly purified components with proper cellular and animal models.

The most relevant example in this area is the blood glucose-lowering protein named γ-conglutin, which affects blood glucose concentrations in animal models at pharmacological doses (Magni *et al.* 2004). This protein has been shown to decrease plasma glucose concentration in a dose-dependent manner. The greatest effect was obtained with γ-conglutin concentrations comparable to half a metformin dose, a well known glucose lowering drug. A wide set of trials with suitable cell models is ongoing. Preliminary results would show a relevant expression/activity alteration of various proteins/enzymes involved in the regulation of glucose homeostasis control. Moreover, clinical trials are currently being carried out to confirm the observed activity in humans with an industrialised γ-conglutin preparation. Since the biological activity of this protein is observed upon oral administration of this protein, several basic questions concerning the absorption and the metabolic fate of the protein may arise. In order to address these topics too, experiments on the transit of the protein through a CaCO cell monolayer are currently being performed.

Although we are still far from having a clear picture on the mechanism of action of this protein, the available findings and those being obtained strongly support the traditional concept of lupin seed as an anti-diabetic food and open the gateway to the exploitation of this protein, mostly as diabetes preventing factor, in proper formulations.

Another example of research work on bioactive proteins from lupin seeds consists in the recent identification of a Bowman-Birk serine-proteinase inhibitor (BBI) (Scarafoni *et al.* 2008), contrary to several former evidences which ruled out the presence of this remarkable protein molecule in this seed. The interest for the proteins of this family arises from their demonstrated effects in a number of pathological situations associated with inflammatory conditions, including cancer, skeletal muscle atrophy, angiogenesis, rheumatoid arthritis, and neurodegenerative and cardiovascular diseases, as well as in radioprotection. Although specific biological studies on the novel lupin BBI have not been carried out yet, also due to the relatively scarce presence of the inhibitor in these seeds, we found interesting that lupin BBI has a peculiar inhibitory pattern as compared to most other inhibitors of this family, being a trypsin/trypsin inhibitor, instead of trypsin/chymotrypsin one. Our former experience in characterising (Ragg *et al.* 2006), cloning and

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expressing a lentil BBI in suitable heterologous hosts (Duranti, unpublished) could help us undertaking this activity, in case the molecular/functional features of lupin BBI would support this strategy.

These molecular/nutraceutical research activities on lupin seed proteins are facilitated by two relatively new achievements. One is the deposition of various amino acid sequences related to the main storage and non-storage lupin seed proteins in bioinformatic databases (Table 1), the other one is the recent publication of the 2D electrophoretic reference map of lupin seed proteins (Magni, *et al.* 2007). As far as the former point is concerned, the deduced amino acid sequences from one or more storage protein genes have shown peculiar features of the lupin proteins with respect to homologous storage proteins from other legume seeds, shown in Table 1. In particular, unusual stretches of glutamate, histidine and arginine residues, consisting of up to 9 consecutive identical residues, were found in the C-terminal region of the α-conglutin acidic subunit as well as the frequent occurrence of twin-arginine residues in the β-conglutin. The molecular meaning of some of this sequence peculiar elements is currently being investigated.

Table 1. Amino acid sequence entries of lupin proteins and their best homologous proteins.

L. albus protein		Greatest alignment (BLAST) score with		Protein name
Conglutin	UniProtKB/ TrEMBL (acc. n.)	UniProtKB/ TrEMBL (acc. n.)	Plant	
A	Q53I54	GLYG3	Soybean	Glycinin G3
		Q9T0P5	Pea	Legumin A
		Q99304	Faba bean	Legumin
B	Q53HY0 and Q6EBC1	Q4LER6	Soybean	β -conglycinin α'subunit
		VCLC	Pea	Vicilin
Γ	Q9FEX1 and Q9FSH9	7SB1	Soybean	7S basic globulin
		Q05929	Carrot	Extra-dermal glycoprotein
		Q8GT67	Tomato	Xylanase inhibitor
Δ	Q333K7	CONG	Peanut	Conglutin
BBI	P85172	B1ACD4	Soybean	Bowman-Birk serine
		Q9AVS2	Pea	proteinase inhibitor

acc. n.: accession number

As far as lupin seed 2D reference map is concerned, 357 protein spots were detected, about 60 of which have been identified by trypsin digestion and mass spectrometry analysis and found to correspond mainly to lupin conglutins. In addition, former studies on the post-translational modifications of these proteins, has allowed us to identify naturally cleaved polypeptide fragments, mostly belonging to the β-conglutin fraction, the extremely modification-prone vicilin-like lupin protein.

The up-loading of the corresponding interactive map on the web is currently being implemented in a dedicated database to provide researchers the opportunity to retrieve specific information on already identified protein components and/or upload newly discovered ones.

The interest in lupin biologically active seed proteins should also include the study of the allergenic potential of these compounds. In this respect, the recent inclusion of lupin in the European list of allergenic food components to be mandatorily declared in the food label is promoting further research in the area. In particular, a number of molecularly-oriented papers have appeared (Magni, C., Ballabio, C. *et al.* 2005, Magni, C., Herndl, A. *et al.* 2005, Foss, N. *et al.* 2006, Goggin *et al.* 2008) and others, dealing with the allergenic potential of specific lupin proteins, are in preparation. While the *in vitro* reactivities of lupin proteins with sensitive patients' sera reflect those of other legume seed major storage proteins, *i.e.* the 7S and 11S globulins, a significantly and specifically reacting protein seems to be the γ-conglutin. It is not extraordinary that the desirable (hypoglycaemic) bioactivity of this protein goes along with its allergenic potential: in both cases the covalent protein structure has to be at least partially preserved during gastro-intestinal transit. A further aspect of interest is the cross-reactivity of peanut-sensitive patients' sera with lupin and other legume seed proteins. Studies on this topic at molecular, cellular and patient level are currently being performed.

The present communication, by providing the state of art of the molecular nutraceutic studies in the area of lupin protein, represents the basis for the development of a scientifically based platform for the current and future perspectives in the value-added optimal exploitation of this still under-exploited, wealthy and healthy protein source for human food.

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