Modern approaches and recent achievements in studying the impact of white lupin seed proteins on human nutrition and health

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Agricultural and Nutritional Aspects of Lupines

Proceedings of the First International Lupine Workshop

Lima - Cuzco, Peru
12 – 21, April 1980

R. Gross and E. S. Bunting

Eschborn 1982

CHEMICAL, NUTRITIONAL AND FUNCTIONAL PROPERTIES OF LUPINE SEED PROTEINS

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Claims on legume seed beneficial properties

American Heart Association (AHA) dietary indications:

…diet should "**contain a variety of foods from all the food categories**"; emphasis should be given to "fruits and vegetables, fat-free and low-fat dairy products, cereal and grain products, legumes and nuts, fish, poultry, and lean meats". (J Nutr 2001;131:132)

Other scientific board claims:

"Grain legumes effectively contribute to a balanced diet and can prevent widely diffused diseases, including type II diabetes and cardiovascular diseases". (Br J Nutr 2002; 88 Suppl 3:239)

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Metabolic fate of dietary proteins

__Established facts:__

- ✓ Dietary proteins are the source of amino acid for the body
- ✓ Proteins are normally degraded to amino acids by proteases during the digestion processes in the gastro-intestinal tract

__Open issues:__

- ✓ Digestion of proteins can be incomplete
- ✓ Full-lenght proteins or their fragments may play various local and systemic effects

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Content of the presentation

- ✓ Do lupin seed proteins have an impact beyond dietary supply of amino acids?
- ✓ How molecular nutraceutics does help in understanding lupin seed protein role
- ✓ Case-studies of selected white lupin seed proteins
- ✓ Concluding remarks

Summary of *Lupinus albus* conglutins' main features

Congl.	***Protein family***	***% of total globulin***	***Native protein***			***Monomer composition***				***Protein function in the seed***
			M_r, kDa	***pI***	***Quaternary structure***	***Subunit name***	***M_r, kDa***	***pI***	***Glycosylation***	
α	11S legumin	35-37	330-430	5.1-5.8	Hexamer	Acidic* Basic*	42-52 20-22	4.5-4.7 6.7-8.6	Yes/No[1] No	Storage
β	7S vicilin	44-45	143-260	5.0-6.0	Trimer	HMW IMW LMW	53-64 25-46 17-20	5.1-5.7 5.3-8.4 4.2-5.0	Yes Yes/No[1] Yes/No[1]	Storage
γ	7S	4-5	200	7.9	Tetramer	Large* Small*	29 17	8.2-8.9 5.8-6.6	Yes No	Unknown
δ	2S	10-12	13	acidic	Monomer (dimer)	Large* Small*	9 4	4.1-4.3 n.d.	No No	Unknown

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TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients

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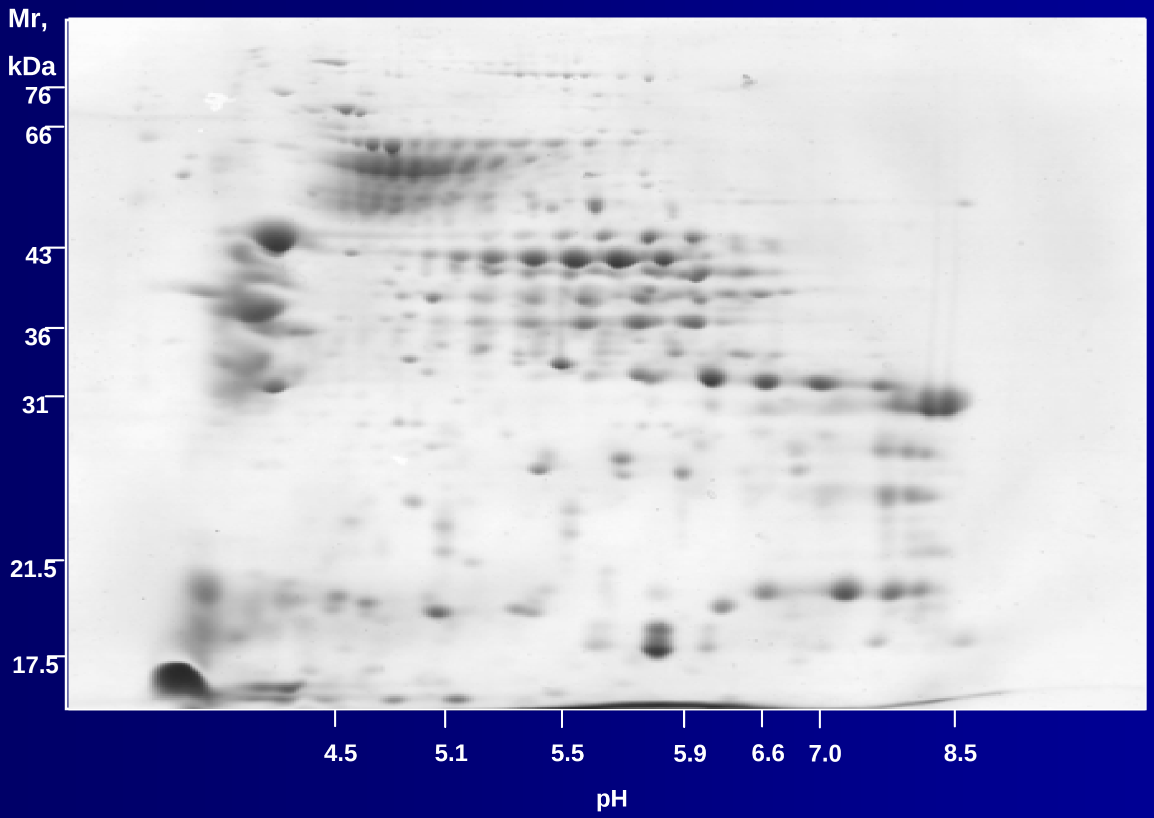
Introduction

The need for plant-derived nutrients is expected to grow due to economic and environmental factors as well as to support the development of new, safe and healthy foods which may respond to the consumers' increasing awareness of the impact of dietary habits on human well-being. Likewise, an increasing demand for plant proteins to be used as food ingredients for the improvement of nutritional, technological and healthy profiles of modern food is emerging, especially in more affluent countries. Although the "historical" role of several legume and cereal grains was as sources of oil for foods, interest in other seed components, including proteins, starches, fibres and minor constituents, is expanding in parallel with industrially available improved separation techniques.

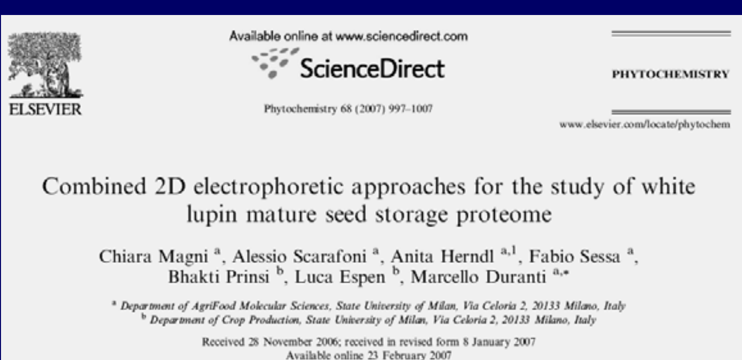
Legume seeds are an abundant source of proteins and, among them, lupin is one of the richest. Indeed, lupin seed deserves greater interest as a result of its chemical composition and augmented availability in many countries in recent years. Lupin is a non-starch leguminous seed with a high protein content, almost as high as that of soybean (about 35% of the dry weight), and a relatively low oil content. Among the lupin seed species, the most cultivated, primarily in Australia, is blue lupin (*Lupinus angustifolius* L.), while the typical European and South American varieties are yellow (*Lupinus luteus* L.) and white (*Lupinus albus* L., Fig. 1) lupins. Blue and yellow lupin seeds are mostly used for feed, while the white lupins are primarily grown for food uses. For this reason, proteins from white lupin seeds will be the focus of this review.

2-D IEF/SDS-PAGE reference map of lupin seed proteome



List of attributed spots in lupin seed proteome

Spot	Vol., %	Protein identification	Accession number	P-value	Score	Mr[a]	MW[b]	pI[a]	pI[b]	A.a. cov. [c], %
10	0.44	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	4.7×10^{-10}	128	63,800	62,032	5.11	6.08	31.1
15	0.23	β−conglutin precursor (*Lupinus albus*)	AAS97865 AJ966470	1.7×10^{-11}	140	64,400	62,130	5.67	6.43	33.8
22	0.64	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	6.4×10^{-11}	170	58,200	62,032	5.20	6.08	36.9
25	0.50	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	3.4×10^{-11}	168	59,400	62,032	5.42	6.08	37.9
29	0.14	β−conglutin precursor (*Lupinus albus*)	AJ966470	2.1×10^{-14}	200	60,883	62,032	5.59	6.08	42.0
32	0.11	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	6.4×10^{-9}	140	56,000	62,032	5.49	6.08	35.0
36	0.18	β−conglu tin precursor (*Lupinus albus*)	AJ966470	2.8×10^{-9}	168	52,900	62,032	5.30	6.08	37.5
41	3.06	No identification				45,400		4.67		
43	0.25	β−conglutin precursor (*Lupinus albus*)	AAS97865 AJ966470	2.2×10^{-9}	100	46,000	62,130	5.80	6.43	25.1
44	0.41	β−congluti n precursor (*Lupinus albus*)	AAS97865	8.1×10^{-11}	98	45,886	62,130	6.13	6.43	21.8
46	1.30	No identification				44,200		4.50		
48	0.37	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	1.9×10^{-10}	180	44,000	62,032	5.40	6.08	41.1
50	1.25	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	6.5×10^{-11}	138	43,500	62,032	5.72	6.08	27.5
52	0.68	β−conglutin precursor (*Lupinus albus*)	AJ966470	2.5×10^{-13}	158	43,562	62,032	6.24	6.08	38.8
53	0.62	No identification				41,900		4.58		
55	0.23	β−conglutin precursor (*Lupinus albus*)	AJ966470	8.4×10^{-13}	150	42,162	62,032	5.70	6.08	37.5
58	0.57	β−conglutin precursor (*Lupinus albus*) alcohol dehydrogenase	AJ966470 AAC62469	7.4×10^{-6} 8.9×10^{-5}	58 28	42,036	62,032 42,241	6.50	6.08 6.09	16.8 8.6
60	3.73	α−conglutin precursor (*Lupinus albus*)	AJ938034	3.3×10^{-7}	50	38,500	60,792	4.58	5.90	13.1
65	0.61	β−conglutin precursor (*Lupinus albus*) α−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865 AJ938034	1.2×10^{-10} 6.7×10^{-8}	88 30	37,000 37,000	62,032 60,792	4.62 4.62	6.08 5.90	22.0 10.9
70	0.47	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.6×10^{-9}	130	37,600	62,032	5.60	6.08	31.1
73	0.63	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.8×10^{-10}	160	37,600	62,032	6.46	6.08	35.4
78	0.59	β−conglutin precursor (*Lupinus albus*)	AJ966470	2.6×10^{-9}	78	34,300	62,032	4.43	6.08	20.9
79	1.86	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.7×10^{-8}	58	34,500	62,032	4.56	6.08	14.1
83	0.42	Putative TAG -associated factor (*Lup inus angustifolius*)	AAN75426	3.5×10^{-4}	10	34,000	32,214	5.68	6.33	5.1
87	1.16	β−conglutin precursor (*Lupinus albus*)	AAS97865	1.2×10^{-11}	110	32,900	62,130	6.59	6.43	24.3
89	1.28	γ-conglutin precursor (*Lupinus albus*)	CAC16394	1.4×10^{-7}	60	30,800	49,21 9	8.65	8.40	18.1
90	1.49	γ-conglutin precursor(*Lupinus albus*) β−conglutin precursor(*Lupinus albus*)	CAC16394 AAS97865 AJ966470	5.4×10^{-9} 1.1×10^{-8}	60 70	30,900 30,900	49,219 62,130	8.85 8.85	8.40 6.43	23.0 17.8
92	0.16	No identification				24,771		5.28		
93	0.30	α−conglutin precursor(*Lupinus albus*)	AJ938034	2.6×10^{-8}	30	23,600	60,792	5.36	5.90	14.1
96	0.20	α−conglutin precursor (*Lupinus albus*)	AJ938034	1.7×10^{-8}	40	23,200	60,792	5.71	5.90	14.4
97	2.17	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.2×10^{-8}	28	20,400	62,032	4.33	6.08	7.2
98	2.37	β−conglutin precursor (*Lupinus albus*)	AAS97865	1.2×10^{-8}	18	18,812	62,130	4.32	6.43	4.7
99	0.48	α−conglutin precursor (*Lupinus albus*) β−conglutin precursor (*Lupinus albus*)	AJ938034 AAS978 65	6.3×10^{-9} 3.7×10^{-7}	20 50	20,200	60,792 62,130	4.96	5.90 6.43	10.7 8.6
101	0.37	small HSP (*Retama raetam*)	AAL32036	1.3×10^{-6}	20	19,900	17,891	5.09	5.82	20.3
102	0.50	18.50kDa class I heat shock protein (*Glicine max*)	P05478	1.2×10^{-6}	40	19,522	18,503	5.34	5.82	21.1
103	0.41	Legumin K (*Pisum sativum*)	S26688	1.5×10^{-5}	10	19,700	56,276	6.67	5.65	2.8
104	0.77	Legumin K (*Pisum sativum*)	S26688	6.9×10^{-5}	6	20,300	56,276	7.09	5.65	2.8
105	1.33	Legumin K (*Pisum sativum*)	S26688	1.5×10^{-7}	10	20,300	56,276	7.85	5.65	2.8
106	0.87	Legumin K (*Pisum sativum*)	S26688	6.5×10^{-6}	4	20,300	56,276	8.3	5.65	2.8
107	0.45	Legumin K (*Pisum sativum*)	S26688	9.8×10^{-6}	8	20,500	56,276	8.57	5.65	2.8
110	0.29	γ-conglutin precursor (*Lupinus albus*)	CAC16394	1.1×10^{-8}	40	18,300	49,219	5.78	8.40	16.8
111	0.65	γ-conglutin precursor (*Lupinus albus*)	CAC16394	7.1×10^{-14}	70	18,900	49,219	6.21	8.40	24.6
112	1.35	γ-conglutin precursor (*Lupinus albus*)	CAC16394	1.2×10^{-7}	40	18,000	49,219	6.19	8.40	15.3
113	0.09	γ-congl utin precursor (*Lupinus albus*)	CAC16394	2.7×10^{-10}	30	18,900	49,219	6.57	8.40	14.4
114	0.17	γ-conglutin precursor (*Lupinus albus*)	CAC16394	6.0×10^{-14}	50	18,200	49,219	6.56	8.40	14.4
115	6.47	δ−conglutin precursor (*Lupinus albus*) β−conglutin precursor (*Lupinus albus*)	CAJ43922 CAJ42100 AJ966470	1.8×10^{-9} 6.0×10^{-8}	30 30	17,300 17,300	17,138 62,032	4.21 4.21	5.47 6.08	26.4 7.2
117	0.38	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.0×10^{-9}	68	16,500	62,032	4.56	6.08	17.7
118	0.69	β−conglutin precursor (*Lupinus albus*)	AJ966470	1.0×10^{-14}	100	16,818	62,032	4.73	6.08	19.6
121	0.21	β−conglutin precursor (*Lupinus albus*)	AJ966470	6.2×10^{-10}	70	16,400	62,032	5.2	6.08	17.9
125	1.00	γ-conglutin precursor (*Lupinus albus*) β−conglutin precur sor (*Lupinus albus*)	CAC16394 AAS97865 AJ966470	1.3×10^{-8} 1.2×10^{-5}	40 20	30,996	49,219 62,032	8.35	8.4 6.08	16.8 5.1
126	0.62	γ-conglutin precursor (*Lupinus albus*) β−conglutin precursor (*Lupinus albus*)	CAC16394 AAS97865 AJ966470	3.8×10^{-9} 1.7×10^{-7}	40 100	32,438	49,219 62,032	8.19	8.4 6.43	13.1 22.3
127	0.89	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	1.6×10^{-13}	110	32,616	62,032	7.60	6.08	24
128	0.89	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	8.2×10^{-12}	100	32,682	62,032	7.08	6.08	22
130	0.27	No identification				26,657		5.62		
131	0.17	No identification				26,333		5.95		
132	0.22	β−conglutin precursor (*Lupinus albus*)	AJ966470 AAS97865	8.6×10^{-5} 1.3×10^{-4}	20 10	26,467	62,032 24,414	6.37	6.08 6.08	22



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Combined 2D electrophoretic approaches for the study of white lupin mature seed storage proteome

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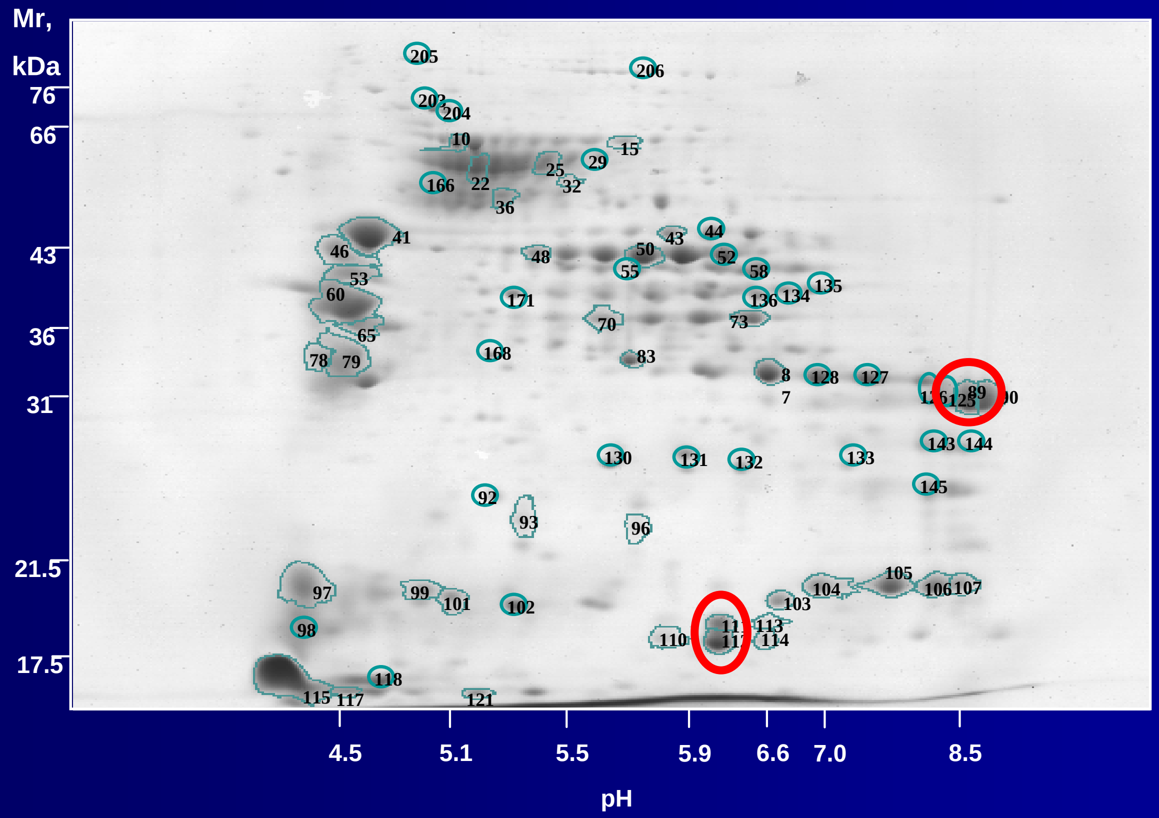
2-D IEF/SDS-PAGE reference map of lupin seed proteome



The original nutraceutical concept

Nutraceutical is “any food, or part of food, considered to provide health benefits, including the prevention and treatment of disease”

Stephen De Felice, 1989

“Let your food be your medicine and let your medicine be your food”

Hippocrates (460-370 B.C.)

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The “molecular nutraceutics” concept

Individual target molecule analyses, involving:

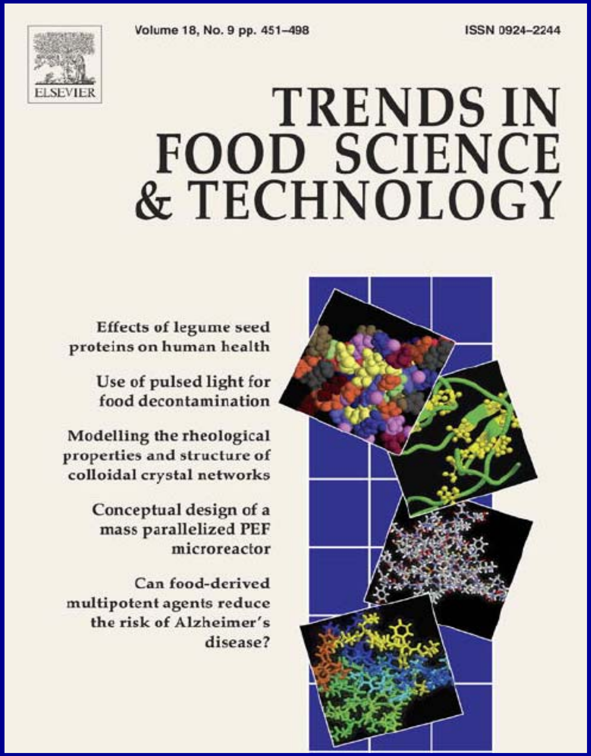
- **Isolation from the wild source**

  (cloning/expression from suitable hosts)

- **Molecular characterisation**

- **Biological activity measurements**

- **Elucidation of the mechanism(s)**



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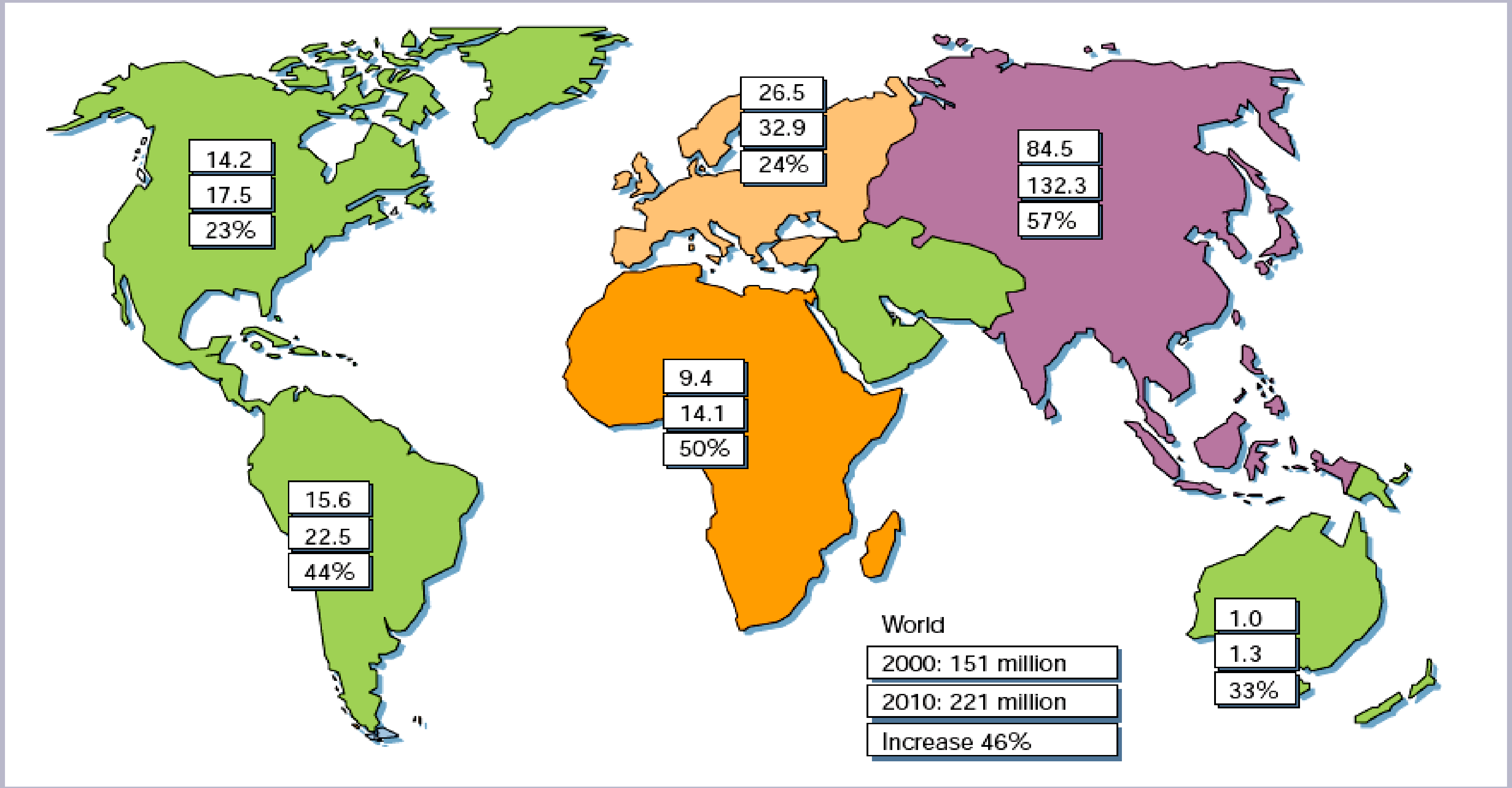
Case study 1:

Blood glucose-controlling lupin protein:

γ-conglutin

Figure 1 Numbers of people with diabetes (in millions) for 2000 and 2010 (top and middle values, respectively), and the percentage increase. Data adapted from ref. 2.



Zimmer, Albert, Shaw NATURE 414 DECEMBER 2001

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Glycemic control

Pulses are low GI foods in force of their starch peculiar composition. Thus, dry legumes are claimed to help glycemic control in diabetic individuals

Role of galattomannan-rich soluble dietary fibre and 4-hydroxyisoleucine (Broca et al., 2000)

α-amylase inhibitors have been claimed to play anti-diabetic effects (McKarty, 2005)

Other causative agents?????

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Binding of lupin γ-conglutin to insulin-agarose

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REVIEWS: CURRENT TOPICS

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THE JOURNAL OF Nutritional Biochemistry

Conglutin γ, a lupin seed protein, binds insulin in vitro and reduces plasma glucose levels of hyperglycemic rats

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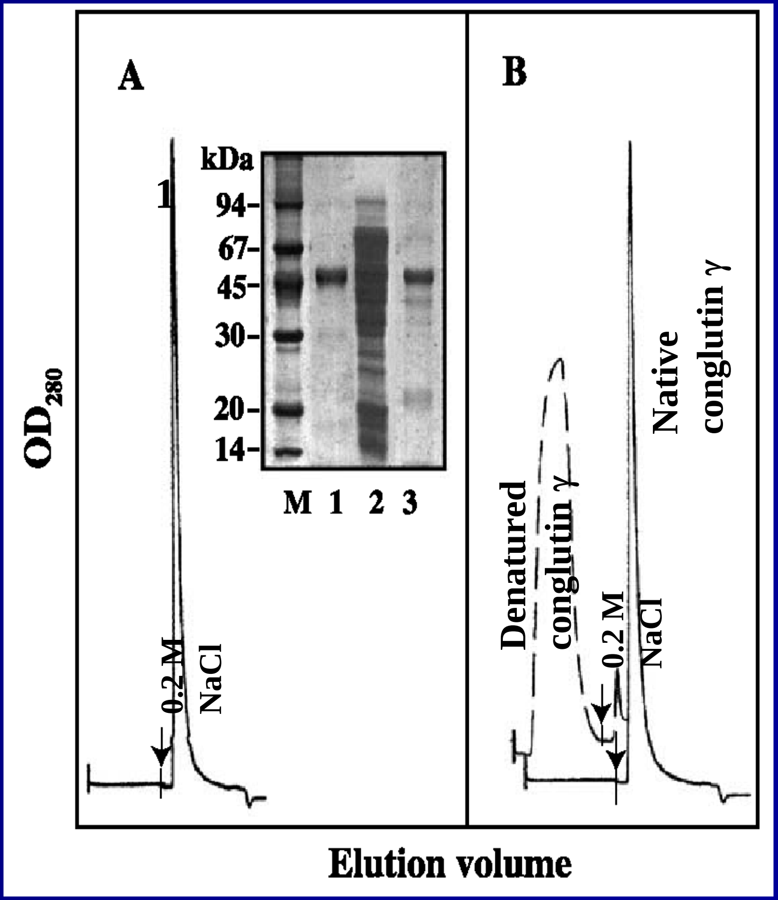
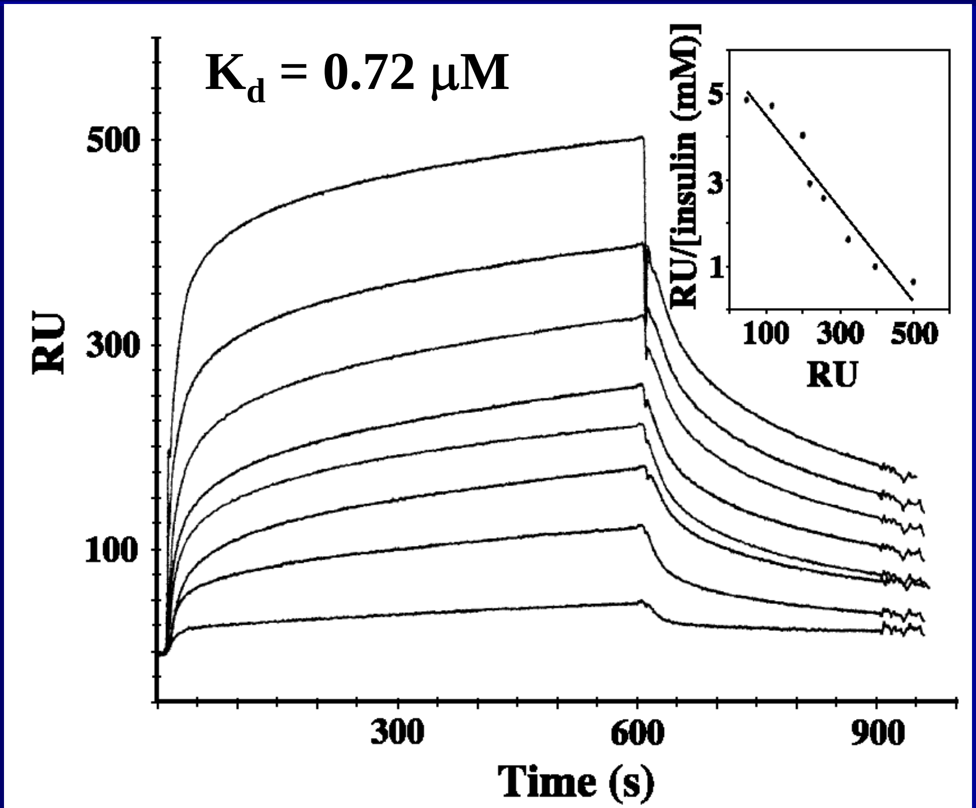
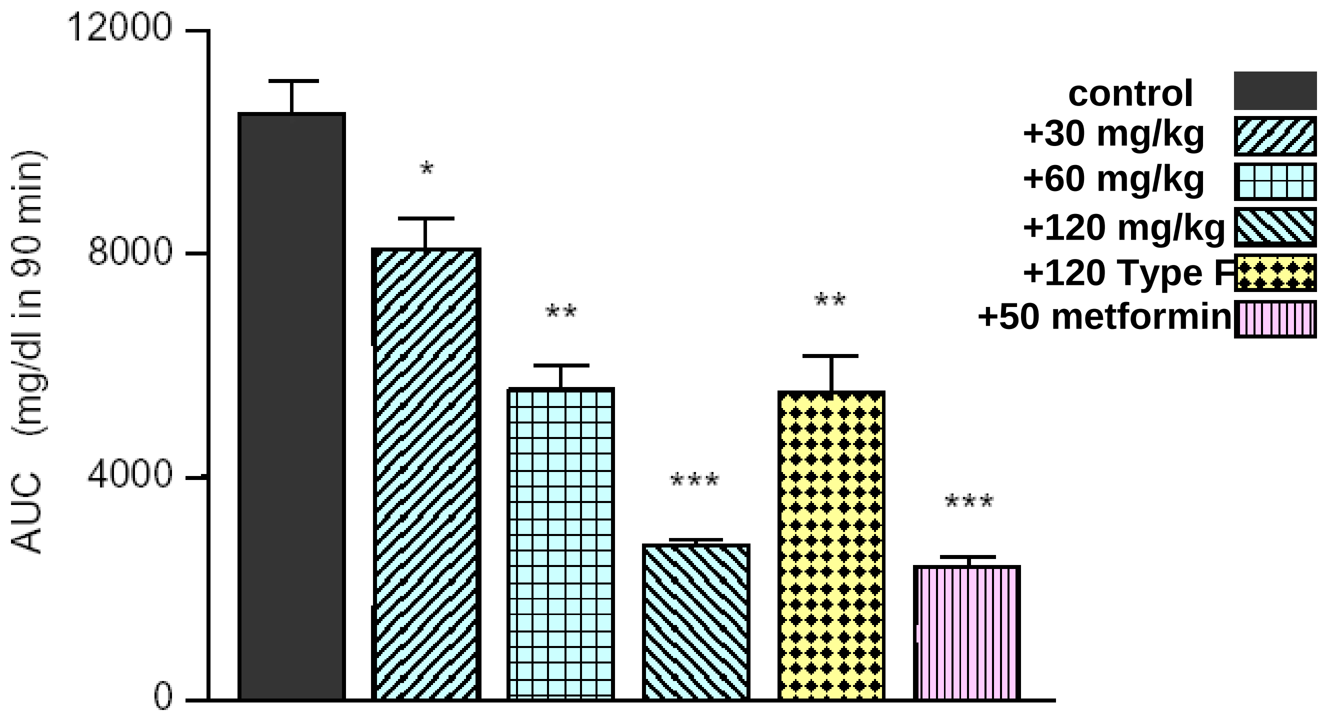


Surface Plasmon Resonance analysis of the interaction between γ-conglutin and insulin



Influence of γ-conglutin on rat plasma glucose concentrations during glucose overloading trials



*: P<0.05, **: P<0.01, ***: P<0.001 vs. control

Alignment of γ-conglutin with wheat xylanase inhibitor, TAXI (30% identity)

```
c32     LYHNSQPTSSSKPNLLVLPIQQDASTKLHWGNILKRTPLMQVPVLLDLNGKHLWVTCSQH 60
TAXI    LPVLAPVTKDPATSLYTIPFHDGAS------------------LVLDVAGPLVWSTCDGG 42
        *   :  *... ..* .:*:::.**                  ::**: *  :* **.

c32     YSSSTYQAPFCHSTQCSRANTHQCFTCTDSTTSRPGCHNNTCGLISSNPVTQESGLGELA 120
TAXI    QPPAEIP---CSSPTCLLANAYPAPGCPAPSCG-SDKHDKPCTAYPYNPVSGACAAGSLS 98
         ..:      * *. *  **:: .  *. .: . .. *:..*   . ***:  .. *.*:

c32     QDVLALHSTHGSKLGSLVKIPQFLFSCAPTFLTQKGLPNNVQGALGLGHAPISLPNQLFS 180
TAXI    HTRFVANTTDGSKPVSKVNVG-VLAACAPSKLLAS-LPRGSTGVAGLANSGLALPAQVAS 156
        :  :. ::*.***  * *::  .* :***: *  . **..  *. **.:: ::** *: *

c32     HFGLKRQFTMCLSSYPTSNGAILFGDINDPNNNNYIHNSLDVLHDMVYTPLTISKQG-EY 239
TAXI    AQKVANRFLLCLPTG--GPGVAIFGGGPVPWP--------QFTQSMPYTPLVTKGGSPAH 206
           : .:* :**.:   . *. :**.   *          :. :.* ****. .  .  :

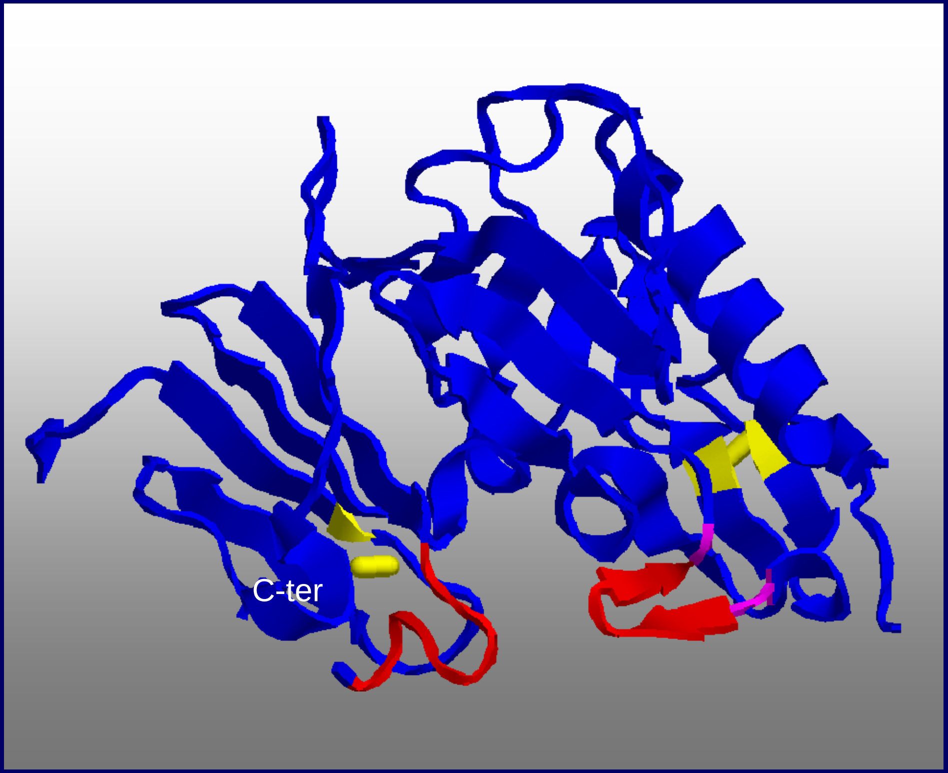
c32     FIQVSAIRVNKHMVIPTKNPSMFPSSSSSSYHESSEIGGAMITTTNPYTVLRHSIFEVFT 299
TAXI    YISARSIVVGDTRVPVPEG-----ALATGGVMLSTRLP-YVLLRPDVYRPLMDAFTKALA 260
        :*.. :* *..  *  .:.     :  ::..   *:.:   ::  .: *  *  .:: :.::

c32     QVFANNVPKQAQVKAVGPFGLCYDTKKISG-----GVPSVDLIMDKSDVVWRISGENLMV 354
TAXI    AQHANGAPVARAVEAVAPFGVCYDTKTLGNNLGGYAVPNVQLGLD-GGSDWTMTGKNSMV 319
          .**..*    *:**.***:*****.:..     .**.*:* :* ..  *  ::*:* **

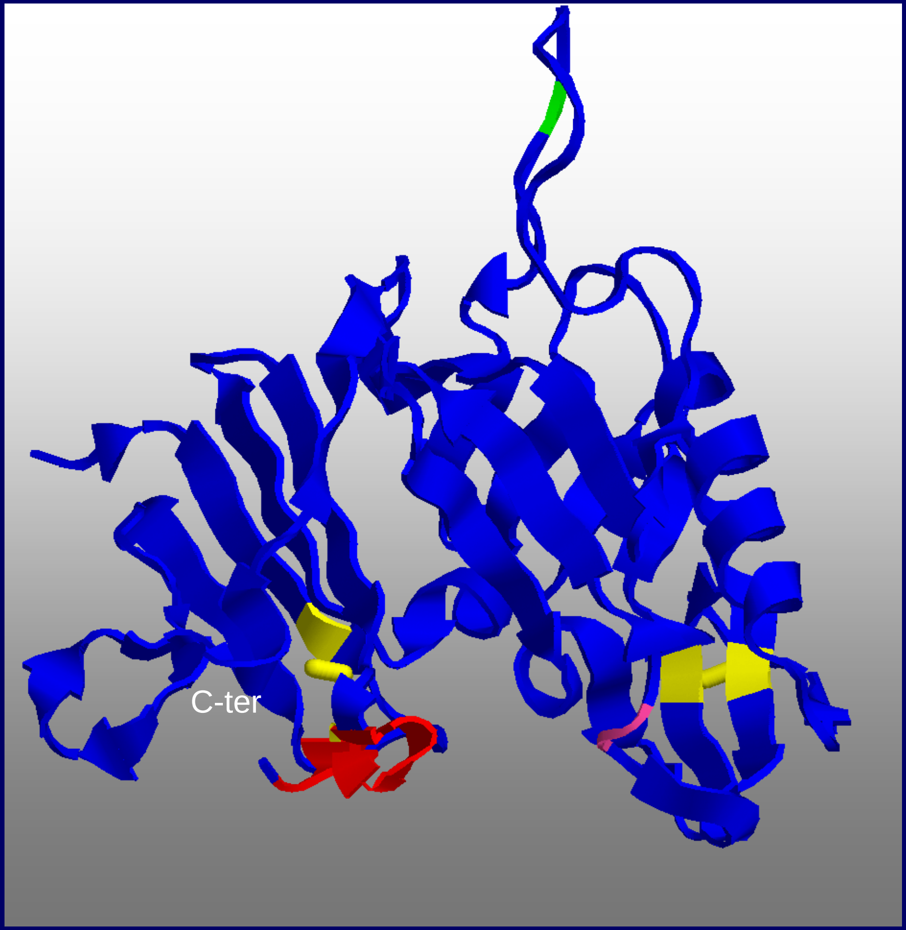
c32     QAQDGVSCLGFVDG-GVHTRAG----IALGTHQLEENLVVFDLARSRVGFNTNSLKSHGK 409
TAXI    DVKQGTACVAFVEMKGVAAGDGRAPAVILGGAQMEDFVLDFDMEKKRLGFSR---LPHFT 376
        :.::*.:*:.**:  ** :  *    : **  *:*: :: **: :.*:**.     .* .

c32     SCSNLFDLNNP 420
TAXI    GCGGL------ 381
        .*..*
```

Comparison of the 3D structures of truncated γ-conglutin and wheat xylanase inhibitor



Truncated TAXI 3D structure
(PDB: 1T6G)



Truncated conglutin γ predictive model
(Swiss-Model Repository)

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Further ongoing studies on γ-conglutin bioactivities

- ✓ Caco2 cell transwell transfer analysis

  (Dr. Alfonso Clemente, CSIC, Granada, Spain)

- ✓ Effect of γ-conglutin on mioblast cell signalling pathways

  (Prof. Livio Luzi, Univ. Milano and San Raffaele Hospital, Milano)

- ✓ Clinical study on healthy and pre-diabetic human volunteers

  (INDENA and Drs. Hancke and Bertoglio, Un. Austral de Chile)

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Case study 2:

Anticarcinogenic (?) lupin protein:

Bowman-Birk Serine Protease Inhibitor

Anti-carcinogenic effects of legume seed components

Compounds that have traditionally been considered beneficial:
- protease inhibitors;
- saponins;
- phytosterols;
- isoflavons;
- phytates;
- fibre and non-digestible starch

α-galactosides: prebiotic molecules that promote in the gut the growth of positive M.O. producing short fatty acids (butyric), protective against colon cancer. Positive M.O. also inhibit pathogen (Gaudard-De Weck, 1999).

Bowman-Birk inhibitor (BBI) family provided evidence to arrest some mammalian tumors (Kennedy, 1998).

Anticancer activities of Bowman-Birk serine proteinase inhibitors from legume seeds

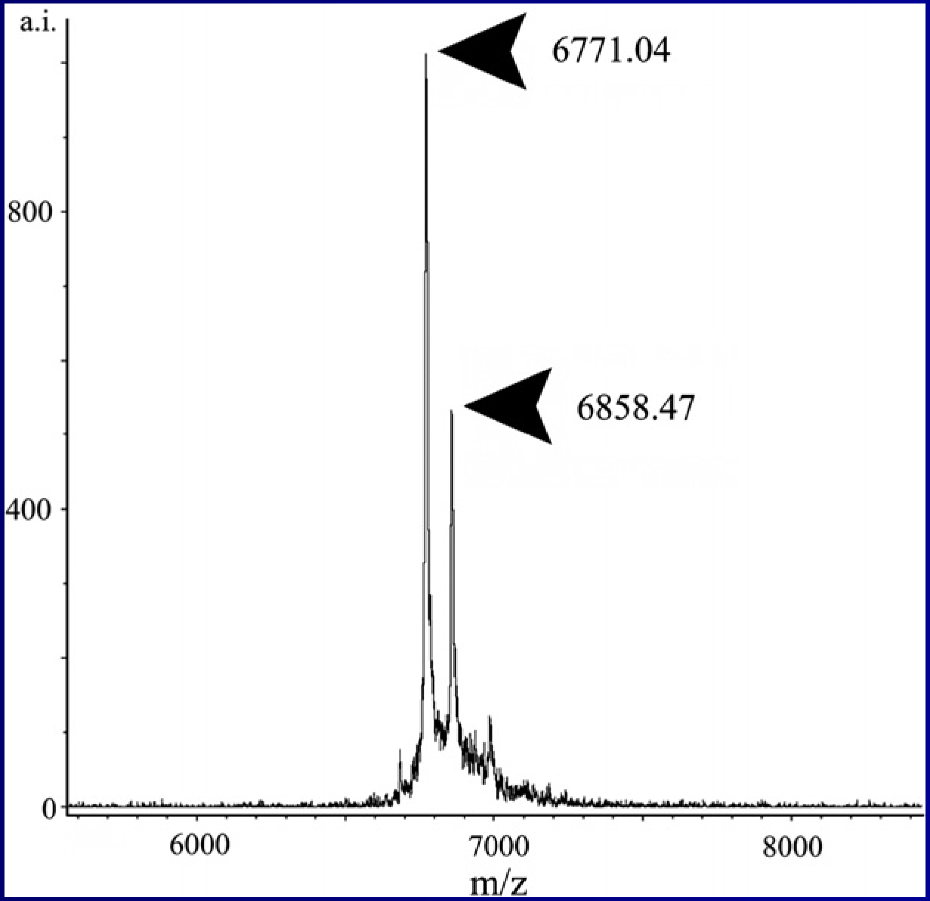
Animal model	Disease type	Carcinogen/ Chemical	Effective Protease inhibitor	Level of effect	Refs
Mouse	Liver cancer	Spontaneous DMH	0.01% BBI in diet 0.1% BBI in diet 0.5% BBI in diet	**100% fewer tumors** **50% fewer tumors** **75% fewer tumors**	[32] [10] [10]
	Colon cancer	DMH	0.1% BBI in diet 0.5% BBI in diet	**50% fewer tumors** **90-100% fewer tumors**	[10, 6] [10, 6]
	Min Mice (Colon cancer)	Genetic susceptibility	0.1% BBI in diet 0.5% BBI in diet	**30% fewer tumors** **40% fewer tumors**	[30] [30]
	Lung cancer	3-MC	10 mg/kg injected BBI	**30% fewer tumors**	[44]
	Human prostate Cancer	Xenograft	1% BBI in diet	**Reduced tumor load**	[40]
	Human pancreas cancer	Xenograft	1 mg/kg/day PCI injected	**70% less tumor volume**	[8]
Rat	Esophagus cancer	MBNA	180 mg BBI tablets	**40% fewer tumors**	[28]
	Mammary gland cancer	Chemical, X-ray	Soybean diet,	**Significant reduction**	[32]
	Irritable bowel disease	Dextrane sulphate	0.5% BBI	**15% less mortality** **significant reduction of symptoms**	[18]
Hamster	Oral cancer	DMBA	1.% BBI in diet 0.001%	**80% fewer tumors** **60% fewer tumors**	[28] [28]
Human	Oral leuko-plakia	Smokers and alcoholics	Daily dose BBI for 1 month	**Reduced neu-oncogene expression**	[41]
	Oral leuko-plakia	Smokers and alcoholics	Daily dose BBI for 1 month	**24% reduced lesion size**	[2]
	PSA	Elder men	Daily dose BBI for 1 month	**Significant reduction (up to 43%)**	[35]
	Prostate volume	Elder men	Daily dose BBI for 1 month	**Significant volume reduction**	[35]

Courtesy of Prof. MA Jongsma, WUR

Purification scheme of BBI from white lupin seeds

Step	μg prot/g seed	TIU/g seed	TIU/mg prot	Purification fold	Protein yield (%)	Activity yield (%)
Aqueous extract	183,100	12,705	69	1.0	100	100
Thermal-treated extract	910	169	186	2.7	0.50	1.32
DEAE-cellulose	148	150	1,029	14.9	0.076	1.18
TAC	4.0	112	28,370	411.1	0.022	0.88

MALDI-TOF mass spectrum of LaBBI



Alignment of legume seed double trypsin BBI amino acid sequences

```
                           1        10        20        30        40        50        60
                           |--------|---------|---------|---------|---------|---------|---
IBB1_LUPAL      -----------SLASKPCCDSCLCTRSIPPQCRCTDIG-ETCHSACKSCICTRSFPPQCRCSDITHFCYKPCTSS-----  63
IBB_MEDSC       ----------TKSTTTACCDFCPCTRSIPPQCQCTDVR-EKCHSACKSCLCTRSFPPQCRCYDITDFCYPSCS-------  62
IBB2_PHAAN      SVHHQDSSDEPSESSHPCCDLCLCTKSIPPQCQCADIRLDSCHSACKSCMCTRSMPGQCRCLDTHDFCHKPCKSRDKD--  78
IBB_PHAAU       ------SHDEPSESSEPCCDSCDCTKSIPPECHCANIRLNSCHSACKSCICTRSMPGKCRCLDTDDFCYKPCESMDKD--  72
IBBD2_SOYBN     --SDQSSSYDDDEYSKPCCDLCMCTRSMPPQCSCEDIRLNSCHSDCKSCMCTRSQPGQCRCLDTNDFCYKPCKSRDD---  75
                                 *** * **  * ** * *       *** *  * **** *  *** *   **   *
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Identification and characterization of a Bowman–Birk inhibitor active towards trypsin but not chymotrypsin in *Lupinus albus* seeds

Alessio Scarafoni [a,*], Alessandro Consonni [a], Valerio Galbusera [a], Armando Negri [b], Gabriella Tedeschi [b], Patrizia Rasmussen [a], Chiara Magni [a], Marcello Duranti [a]

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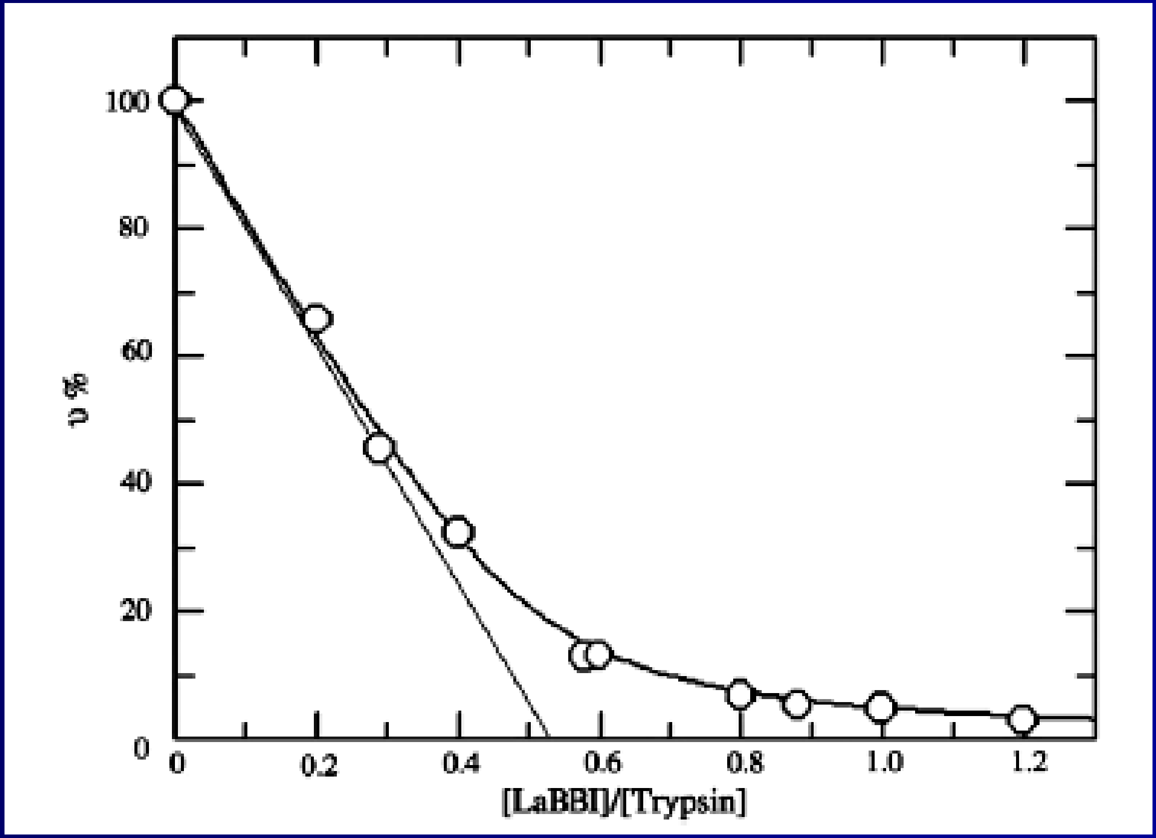
Titration curve of LaBBI with trypsin



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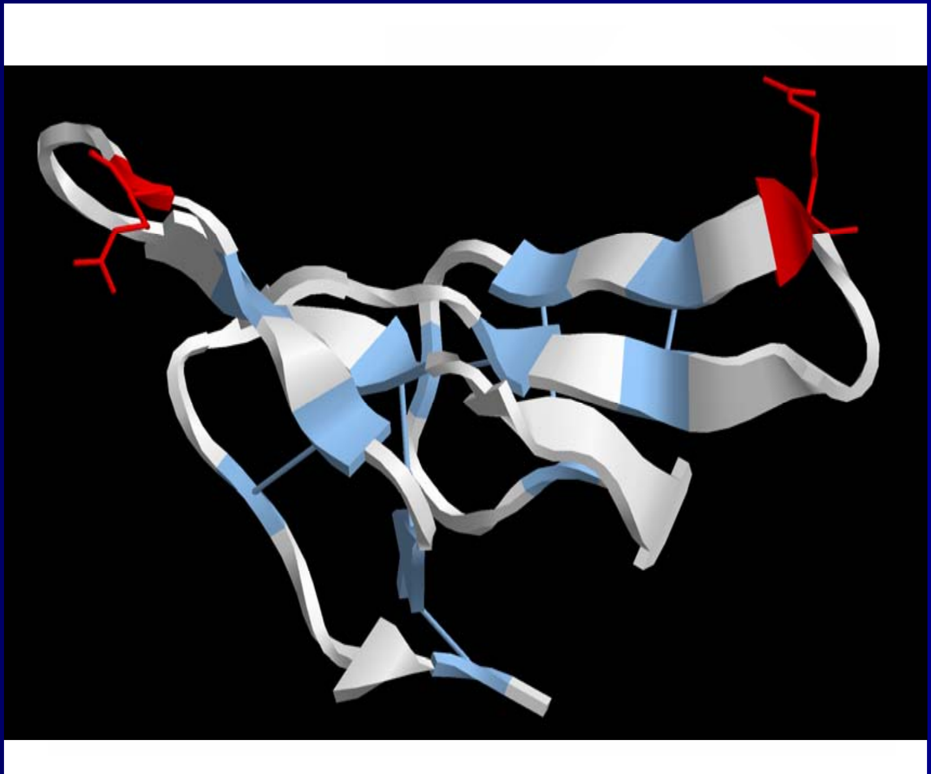
Predicted 3D structure of LaBBI



Based on snail medic seed (Med. scutellata, L.) BBI structure (1mvz):

Seq. Id.: 78%

E value: 2.6 x 10^{-10}

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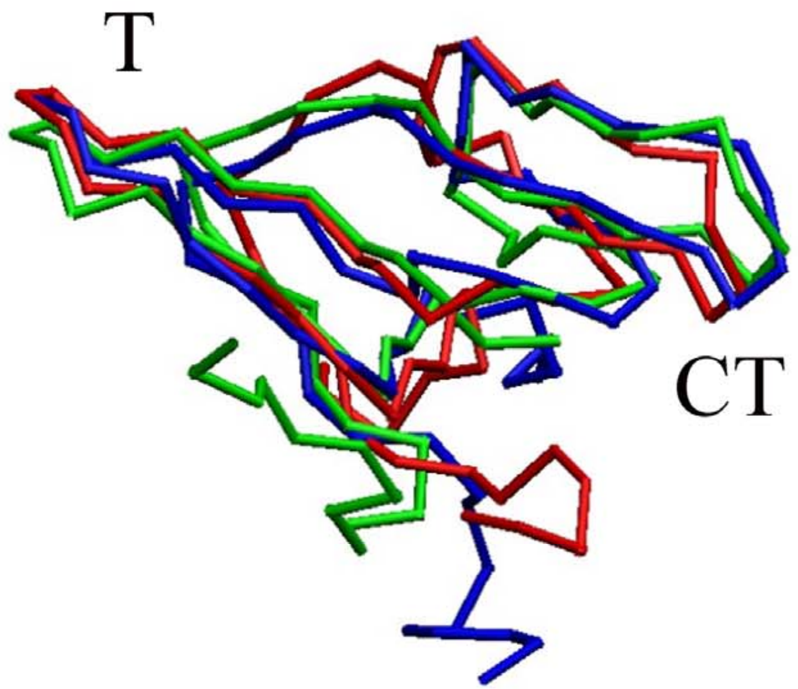
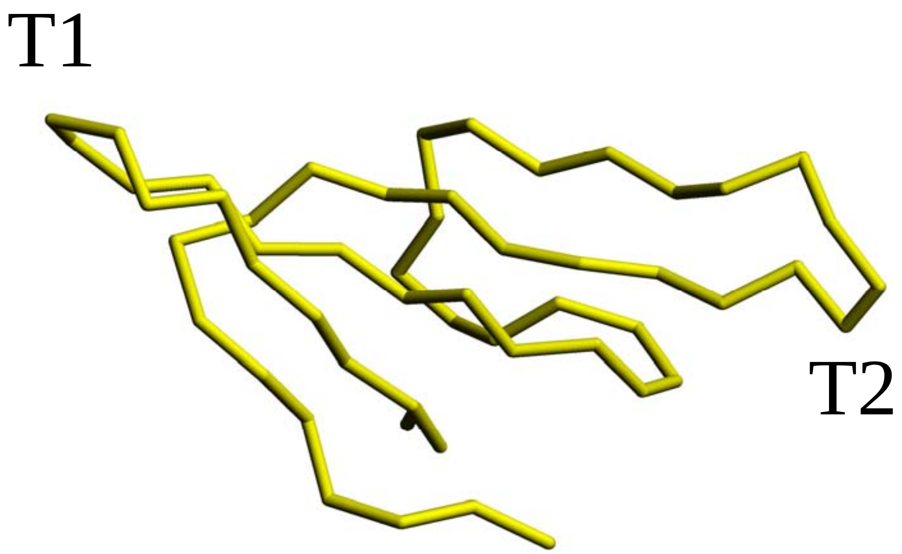
Comparison of the 3D structures of lentil (2AIH), pea (1PBI), soybean (1BBI) with lupin BBI





Examples of biologically active legume seed proteins

Protein family	*Examples*	*Type of protein*	*Source*	*Adverse activity*	*Beneficial activity*
Proteinase inhibitors	**BBI**	**Small, compact**	**Many seeds**	**Inhibition of endogenous proteolytic enzymes**	**Anticarcinogenic, anti-inflammatory**
Amylase inhibitors	**2S albumin**	**Small**	**Many seeds**	**Inhibition of endogenous amylolytic enzymes**	**Anti-obesity, diabetes, metabolic syndrome**
Storage proteins	**Conglycinin α' subunit**	**Large chain**	**Soybean**	**Not applicable**	**Hypolipidemic**
	Gamma conglutin	**Large chain**	**Lupin**	**Not applicable**	**Hypoglycemic**
	Vicilin	**Fragment**	**Lupin**	**Not applicable**	**Lectin**
Lectins	**PHA**	**Medium size**	**Many legume seeds**	**Hemagglutination**	**Immuno-modulation**
Peptides	**ACEIn**	**Small**	**Few seeds**	**Not known**	**Hypotensive**
Thiamine BP	**PA2**	**Small**	**Many seed**	**Not applicable**	**Vitamin vehiculation**

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Currently available patents related to lupin seed protein nutraceutics

- THE USE OF LUPIN CONGLUTIN FOR THE TREATMENT OF TYPE II DIABETES (WO2004071521)
- A RECENTLY SUBMITTED ITALIAN PATENT ON THE INSULIN-MIMETIC ACTIVITY OF γ-CONGLUTIN
- PASTA ENRICHED WITH VEGETABLE PROTEINS (EP0997078)
- A NUMBER OF PATENTS ON DEMONSTRATED EFFECTS OF BBIs

Concluding remarks

1. Molecular nutraceutics (of food proteins) is an intrinsically reductionist approach…

…however, system simplyfication is required for:

- ❖ **identifying** the responsible molecules
- ❖ **understanding** the effects and mechanisms
- ❖ **optimising** the exploitation strategies

2. Synergies and antagonisms between components are likely to occur in the whole food systems

3. Diseases involved are generally **multifactorial**

4. Many fundamental aspects are still open and require **basic research**

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