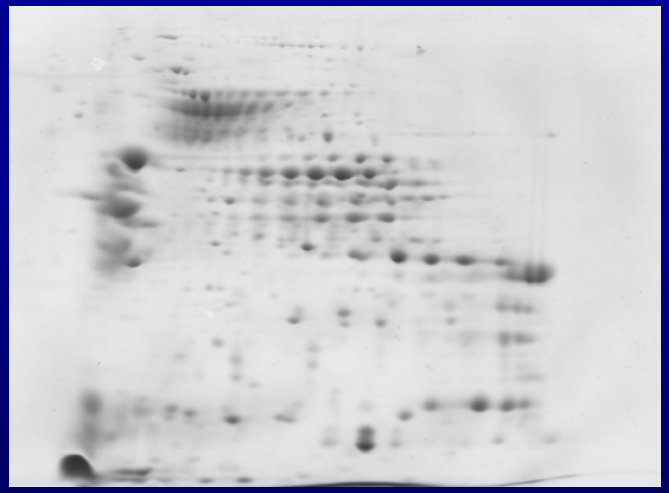
The potentialities of proteomic analysis and use of antibodies in lupin based-food safety assessement and traceability

Marcello Duranti
Department of Agrifood Molecular Sciences
University of Milano, Italy

Getting 2D IEF/SDS-PAGE molecular picture of a biological system

White lupin cotyledonary proteins



Soybean cotyledonary proteins

Content of the presentation

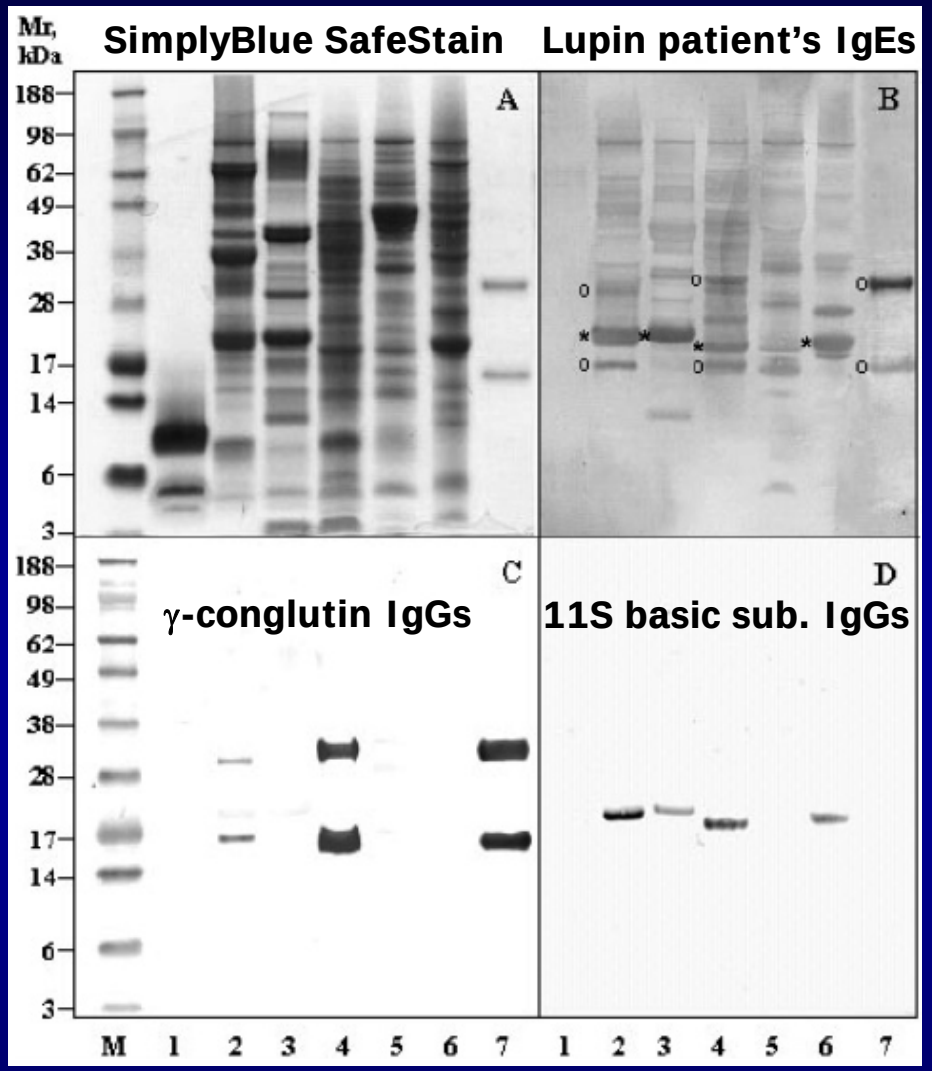
- Applications to allergen identification
- Applications to industrial process evaluation
- Specific use of antibodies
- Conclusions

EU Directive including lupin in the list of ingredients which are likely to cause adverse reactions in susceptible individuals

L 368/110 EN Official Journal of the European Union 23.12.2006

COMMISSION DIRECTIVE 2006/142/EC of 22.12.2006 amending Annex IIIa of Directive 2000/13/EC of the European Parliament and of the Council listing the ingredients which must under all circumstances appear on the labelling of foodstuffs

1D electrophoresis and Western blot analysis of selected legume seed protein extracts



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

One- and Two-Dimensional Electrophoretic Identification of IgE-Binding Polypeptides of *Lupinus albus* and Other Legume Seeds

Chiara Magni,[†] Anita Herndl,[#] Elena Sironi,[†] Alessio Scarafoni,[†] Cinzia Ballabio,[‡] Patrizia Restani,[‡] Roberto Bernardini,[§] Elio Novembre,[§] Alberto Vierucci,[§] and Marcello Duranti*,[†]

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Assessement of the immuno-cross reactivities of specific polypeptides in peanut (A), soybean (B) and lupin (C) 2D electrophoretic maps

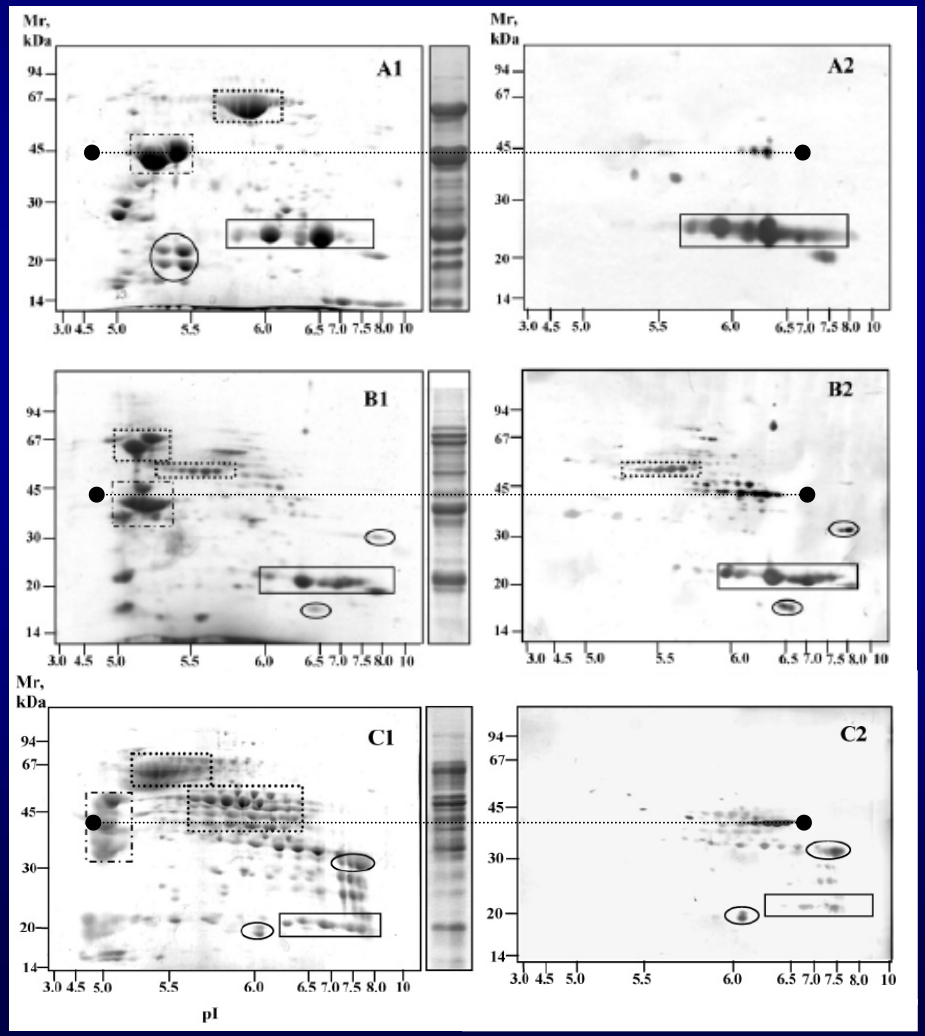
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2005**, *53*, 2275–2281

Two-Dimensional Electrophoresis and Western-Blotting Analyses with anti Ara h 3 Basic Subunit IgG Evidence the Cross-Reacting Polypeptides of *Arachis hypogaea*, *Glycine max*, and *Lupinus albus* Seed Proteomes

Chiara Magni,[†] Cinzia Ballabio,[‡] Patrizia Restani,[‡] Elena Sironi,[†] Alessio Scarafoni,[†] Claudio Poiesi,[§] and Marcello Duranti*,[†]

Department of Agrifood Molecular Sciences, University of Milan, via Celoria 2, 20133 Milano, Italy, Department of Pharmacological Sciences, University of Milan, via Balzaretti 9, 20133 Milano, Italy, and Institute of Microbiology, Spedali Civili, P.le Spedali Civili, 1, 25123 Brescia, Italy

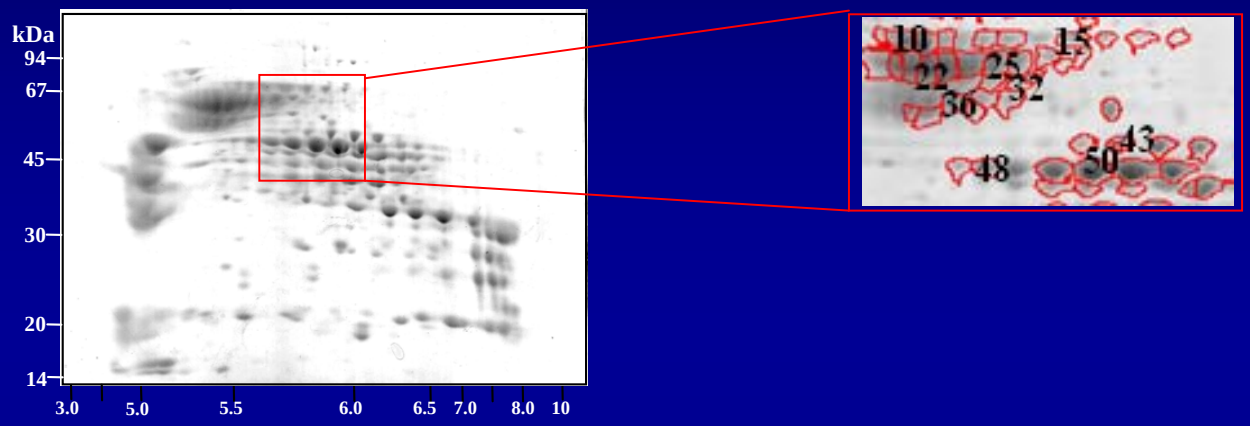


Aminoacid sequence alignments of lupin, soybean and peanut 11S globulin basic subunits

```
tr|Q53I54|Q53I54_LUPAL      GLEETLCTMKLRHNIGESTSPDAYNPQAGRFKTLTSIDFPILGWLGLAAE 50
sp|P04776|GLYG1_SOYBN       GIDETICTMRLRHNIGQTSSPDIYNPQAGSVTTATSLDFPALSWLRLSAE 50
tr|A1E2B0|A1E2B0_ARAHY      GLIETVCTLRLVHQLAESTDADKYNPRAG---------F--LT--ALT-- 35
                            *: **:**::* *:.:::::.* ***:**         *  *    *:

tr|Q53I54|Q53I54_LUPAL      HGSIYKNALFVPYYNVNANSILYVLNGSAWFQVVDCSGNAVFNGELNEGQ 100
sp|P04776|GLYG1_SOYBN       FGSLRKNAMFVPHYNLNANSIIYALNGRALIQVVNCNGERVFDGELQEGR 100
tr|A1E2B0|A1E2B0_ARAHY      --PLT----F-PSFNMSN---L-AL--IDVFSTECCDG-----ATLH--- 64
                              .:     * * :*:.    : .*     :..  *.*      . *:

tr|Q53I54|Q53I54_LUPAL      VLTIPQNYAAAIKSLSDNFRYVAFKTNDIPQIATLAGANSEISALPLEVV 150
sp|P04776|GLYG1_SOYBN       VLIVPQNFVVAARSQSDNFEYVSFKTNDTPMIGTLAGANSLLNALPEEVI 150
tr|A1E2B0|A1E2B0_ARAHY      --------------------------------------------------

tr|Q53I54|Q53I54_LUPAL      AHAFNLNRDQARQLKNNNPYKFLVPPPQSQLRAVA 185
sp|P04776|GLYG1_SOYBN       QHTFNLKSQQARQIKNNNPFKFLVPPQESQKRAVA 185
tr|A1E2B0|A1E2B0_ARAHY      ------------------------------------
```

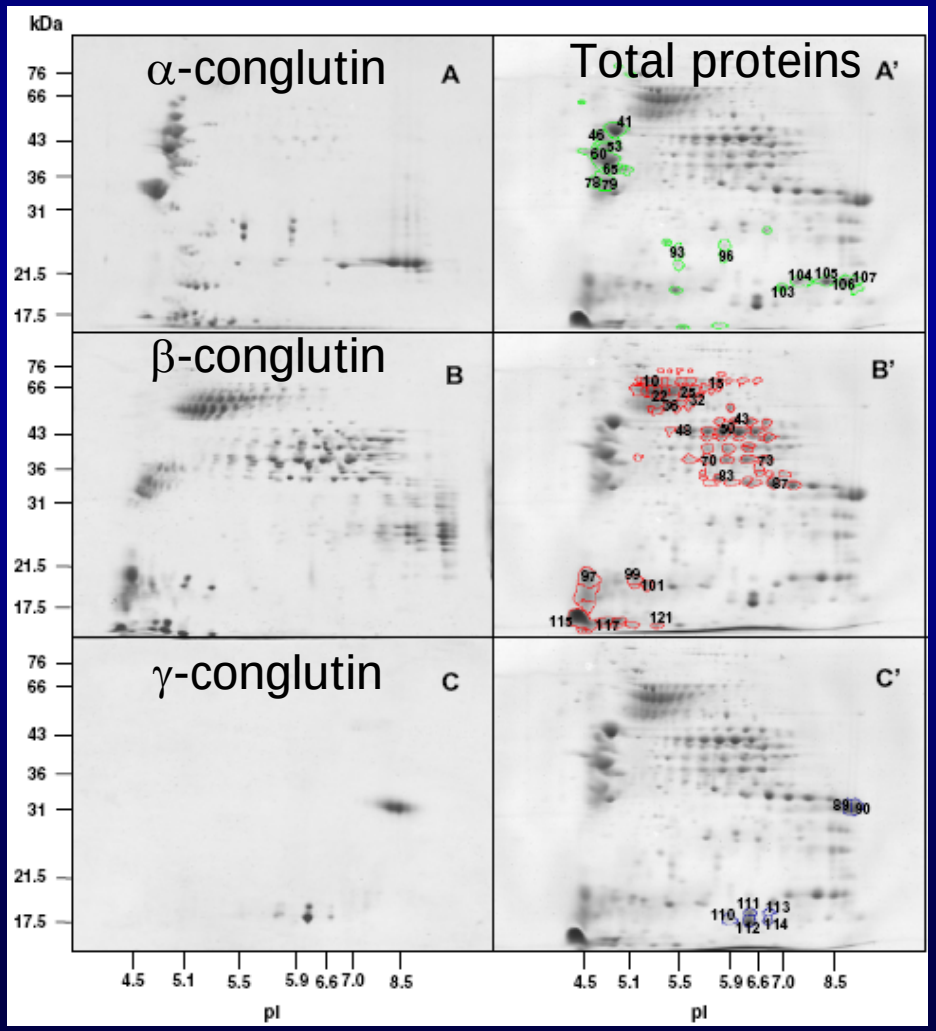
SeqA	Name	Len(aa)	SeqB	Name	Len(aa)	Score
1	tr|Q53I54|Q53I54_LUPAL	185	2	sp|P04776|GLYG1_SOYBN	185	65
1	tr|Q53I54|Q53I54_LUPAL	185	3	tr|A1E2B0|A1E2B0_ARAHY	64	35
2	sp|P04776|GLYG1_SOYBN	185	3	tr|A1E2B0|A1E2B0_ARAHY	64	31

Canonical proteomic approach



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	4.7×10^{-10}	128	63,800	62,032	5.11	6.08	31.1
15	0.23	β-conglutin precursor (*Lupinus albus*)	AAS97865	1.7×10^{-11}	140	64,400	62,130	5.67	6.43	33.8
22	0.64	β-conglutin precursor (*Lupinus albus*)	AJ966470	6.4×10^{-11}	170	58,200	62,032	5.20	6.08	36.9
25	0.50	β-conglutin precursor (*Lupinus albus*)	AJ966470	3.4×10^{-11}	168	59,400	62,032	5.42	6.08	37.9
32	0.11	β-conglutin precursor (*Lupinus albus*)	AJ966470	6.4×10^{-9}	140	56,000	62,032	5.49	6.08	35.0
36	0.18	β-conglutin 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	2.2×10^{-9}	100	46,000	62,130	5.80	6.43	25.1
46	1.30	No identification				44,200		4.50		
48	0.37	β-conglutin precursor (*Lupinus albus*)	AJ966470	1.9×10^{-10}	180	44,000	62,032	5.40	6.08	41.1
50	1.25	β-conglutin precursor (*Lupinus albus*)	AJ966470	6.5×10^{-11}	138	43,500	62,032	5.72	6.08	27.5

Group comparative analysis of lupin 2D maps with purified globulin fractions



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PHYTOCHEMISTRY
ELSEVIER
Phytochemistry 68 (2007) 997–1007
www.elsevier.com/locate/phytochem

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

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[b] Department of Crop Production, State University of Milan, Via Celoria 2, 20133 Milano, Italy

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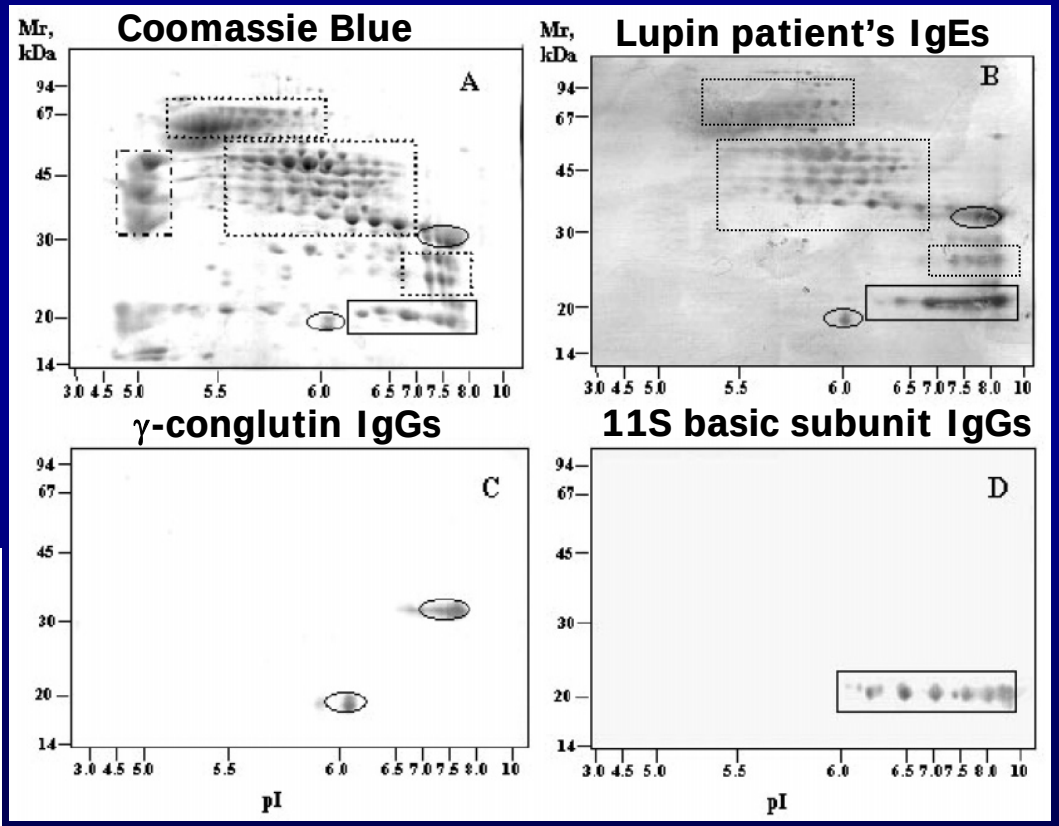
Available online 23 February 2007

2D electrophoretic identification of lupin protein potential allergens



JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

One- and Two-Dimensional Electrophoretic Identification of IgE-Binding Polypeptides of *Lupinus albus* and Other Legume Seeds

CHIARA MAGNI,[†] ANITA HERNDL,[#] ELENA SIRONI,[†] ALESSIO SCARAFONI,[†] CINZIA BALLABIO,[‡] PATRIZIA RESTANI,[‡] ROBERTO BERNARDINI,[§] ELIO NOVEMBRE,[§] ALBERTO VIERUCCI,[§] AND MARCELLO DURANTI*[,†]

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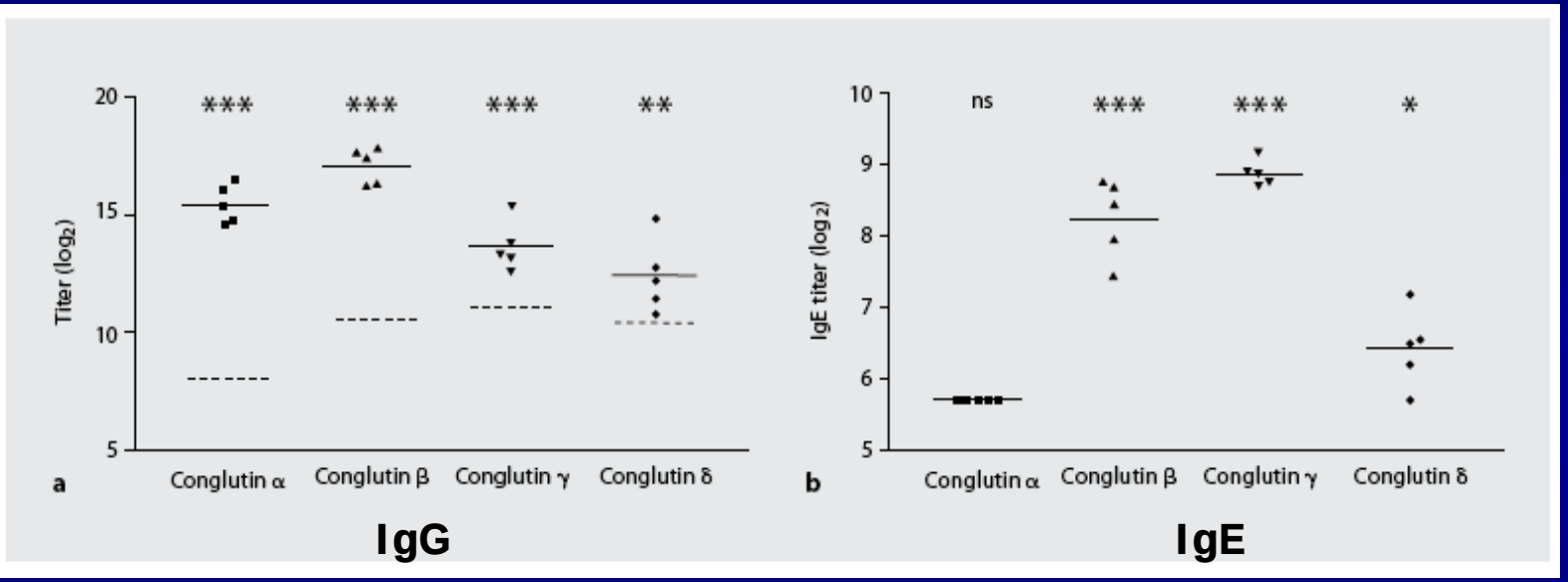
ILC, Perth 2008

Titers of specific IgGs and IgEs in the cholera toxin mouse model



International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2006;141:141–150
DOI: 10.1159/000094716

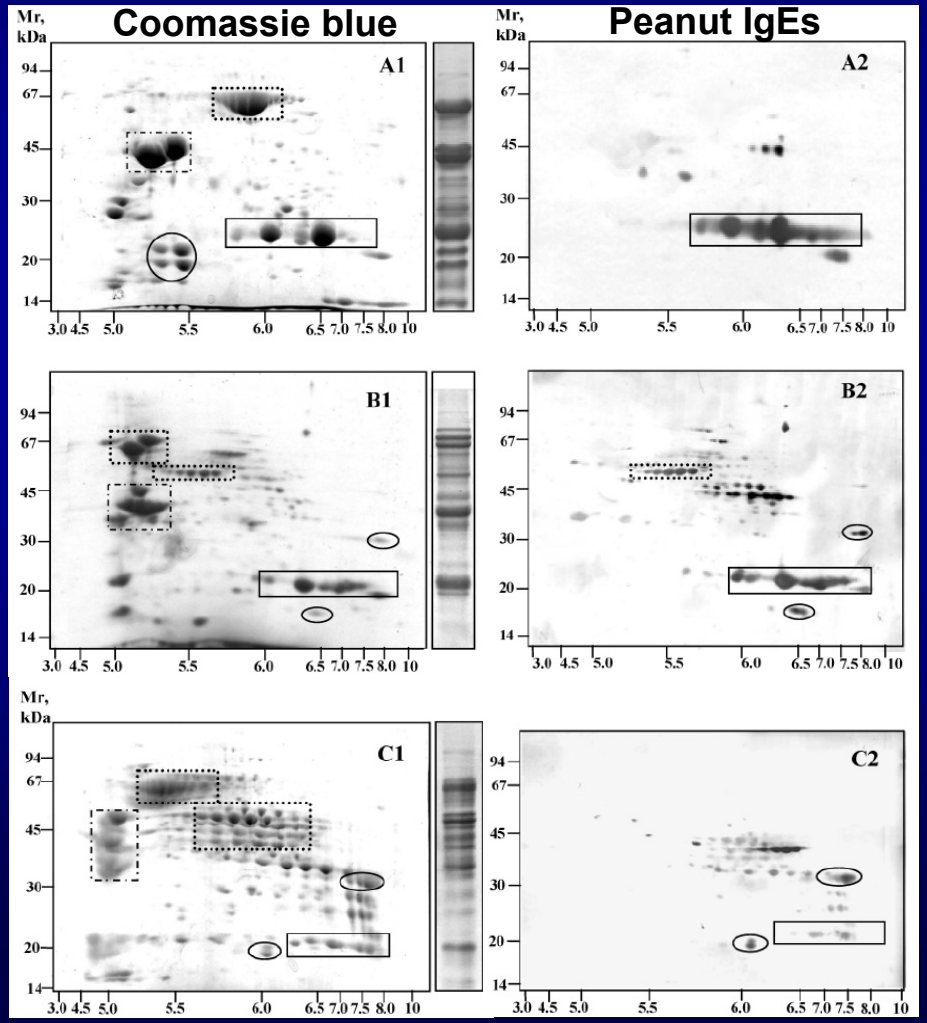
Assessment of Lupin Allergenicity in the Cholera Toxin Model: Induction of IgE Response Depends on the Intrinsic Properties of the Conglutins and Matrix Effects

Nicolai Foss[a] Marcello Duranti[b] Chiara Magni[b] Hanne Frøkiær[a]

[a]Biochemistry and Nutrition Group, BioCentrum-DTU, Technical University of Denmark, Kgs. Lyngby, Denmark; [b]Dipartimento di Scienze Molecolari Agroalimentari, Università degli Studi di Milano, Milano, Italy

Mice fed on a soybean-free diet were fed lupin flakes for 5 weeks

2D electrophoretic analysis of the cross-reactivities between peanut (A), soybean (B) and lupin (C) polypeptides with peanut IgEs



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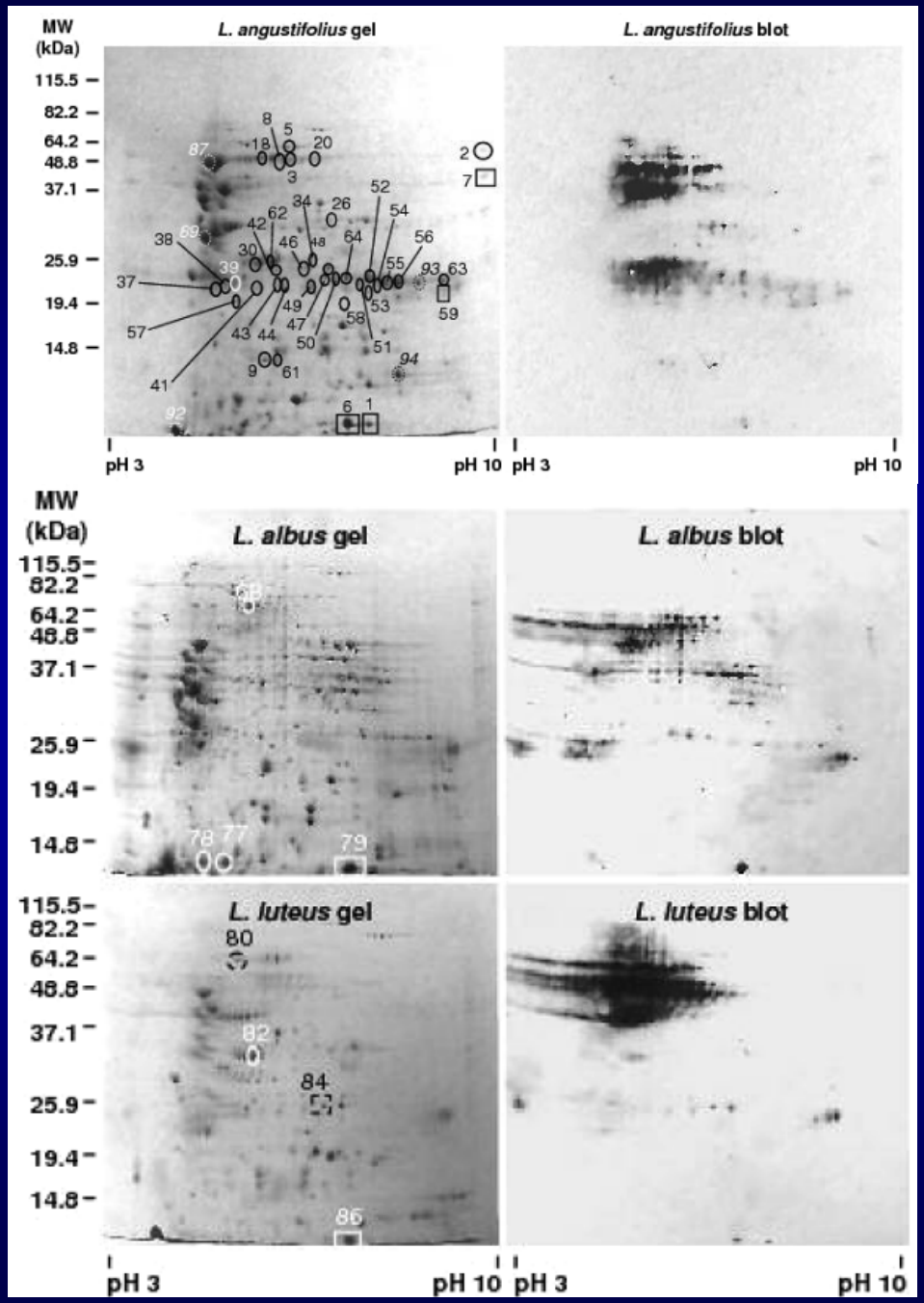
2D electrophoretic analysis and immuno reaction with lupin-sensitized patient sera of three lupin species



Total proteins Patient sera

J. Agric. Food Chem. 2008, 56, 6370–6377

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Proteomic Analysis of Lupin Seed Proteins To Identify Conglutin β as an Allergen, Lup an 1

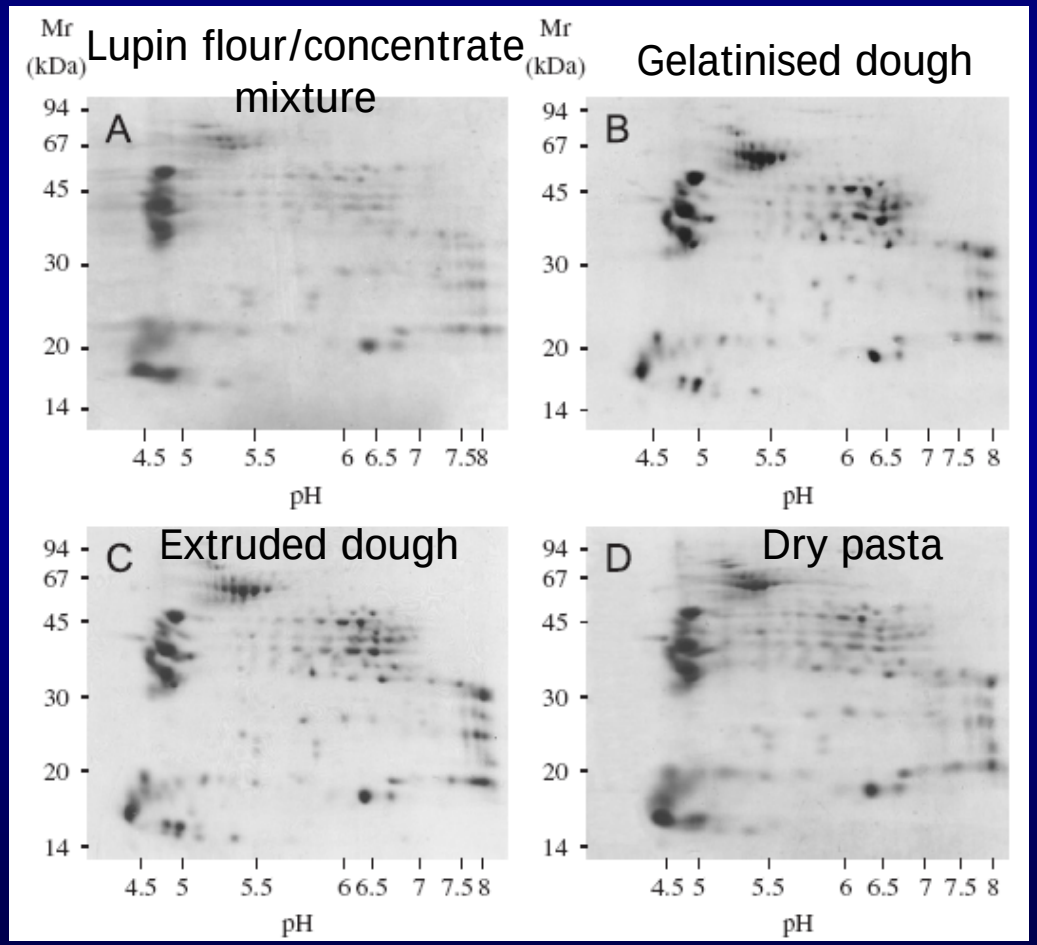
DANICA E. GOGGIN,† GISELA MIR,‡ WILLIAM B. SMITH,§ MARTIN STUCKEY,|| AND PENELOPE M. C. SMITH*,‡

School of Plant Biology, University of Western Australia, 35 Stirling Highway, Crawley, Western Australia 6009, Australia, School of Biological Sciences, University of Sydney, New South Wales 2006, Australia, Clinical Immunology and Allergy, Royal Adelaide Hospital, North Terrace, South Australia 5000, Australia, and Clinical Immunology and Allergy, St. John of God Pathology, Hollywood Private Hospital, Monash Avenue, Nedlands, Western Australia 6009, Australia

Industrial process proteomics:

lupin-based pasta production on line analysis

Plada, Italy (Heinz group)



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ScienceDirect
LWT 41 (2008) 1011–1017
LWT
www.elsevier.com/locate/lwt
ELSEVIER

Application of two-dimensional electrophoresis to industrial process analysis of proteins in lupin-based pasta

Jessica Capraro[a], Chiara Magni[a], Massimo Fontanesi[b], Andrea Budelli[b], Marcello Duranti[a,*]

[a]*Dipartimento di Scienze Molecolari Agroalimentari, Università Degli Studi di Milano, Via Celoria, 2, I-20133 Milano, Italy*
[b]*PLADA Industries srl, Via Migliara 45, Borgo Grappa, Latina, Italy*

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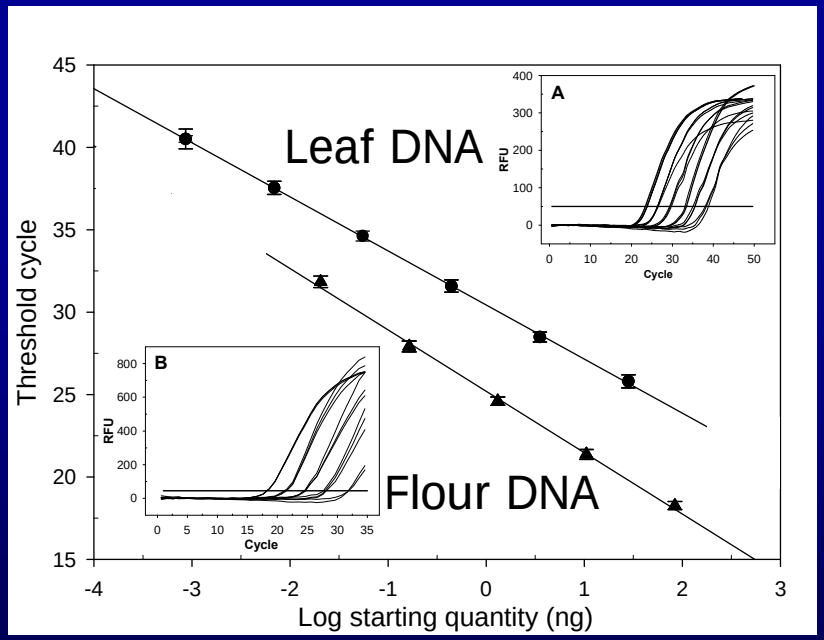
ILC, Perth 2008

Coming soon lupin detection ELISA kit from R-Biopharm (D)

Some features:

- pAbs to γ-conglutin
- specificity: cross-reactivity with soybean Bg7S
- sensitivity: 1-5 ppm

Real-time PCR for lupin DNA detection in foods



Specificity of cγ32-f5/cγ32-r3 primer pairs

Sample		PCR amplification
Lupin	*Lupinus albus*	+
Soybean	*Glycine max*	-
Corn	*Zea mays*	-
Soft wheat	*Triticum aestivum*	-
Durum wheat	*Triticum durum*	-
Chicken egg	*Gallus gallus*	-
Bovine milk	*Bos taurus*	-

Acknowledgements

DISMA team:

Jessica Capraro
Alessandro Consonni
Chiara Magni
Alessandro Ronchi
Alessio Scarafoni
Fabio Sessa