



Molecular characterization and allergenicity assessment of novel protein ingredients

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ProxiMed



PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION
IN THE MEDITERRANEAN AREA

17 Partners

10 Countries

8 Work packages

*“Exploration and Implementation of Products with Alternative Proteins
in Mediterranean Region”*



our role in WP6

PROTEIN CHARACTERIZATION

Protein content

Protein profile

Protein quality
(Amino acids)

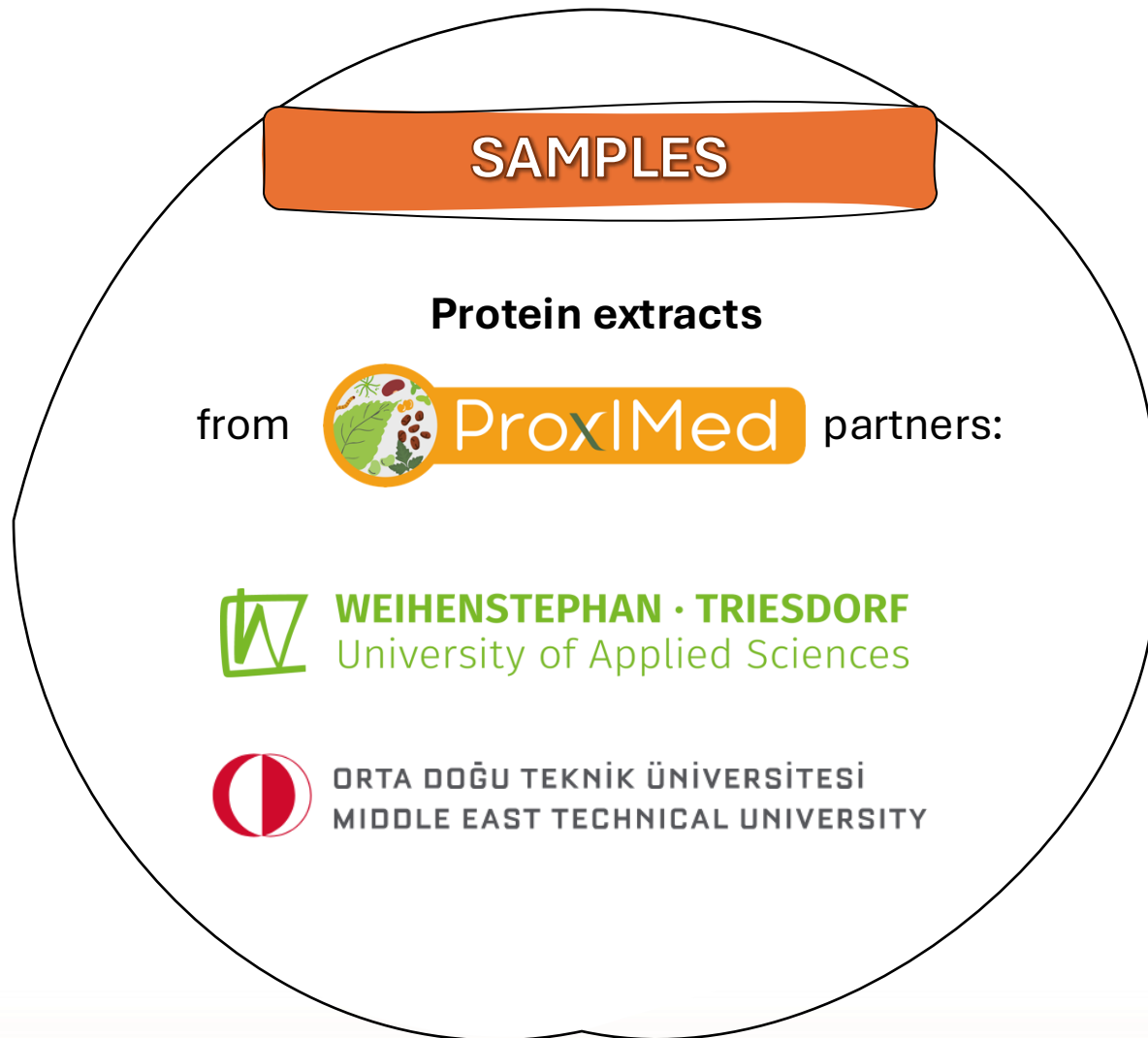
Protein digestibility

ALLERGENICITY POTENTIAL ASSESSMENT

**Possibility of adverse reactions
in sensitized patients**



ProxiMed



“Novel foods” sources:
Duckweed



**Traditional
alternative sources:**
Faba bean



Agro-industrial by-products:
Tomato pomace



Protein characterization

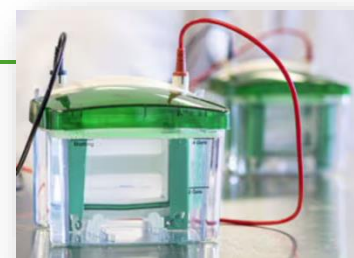
Kjeldahl
method

Total protein
content analysis



SDS-PAGE

Protein profile
analysis



UPLC-MS

Amino acids analysis:
(essential AAs,
actual conversion
factor, AA score)



Allergenicity potential assessment



In vitro
immunoblotting
assay

In silico
analysis

Possibility of adverse reactions
in sensitized patients

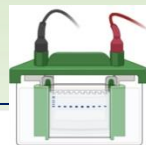
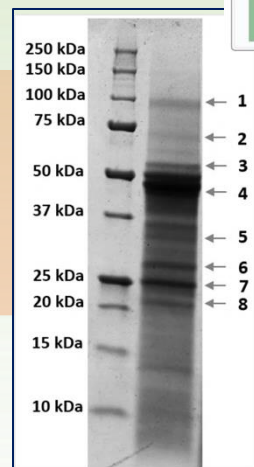


Allergenic potential of alternative proteins

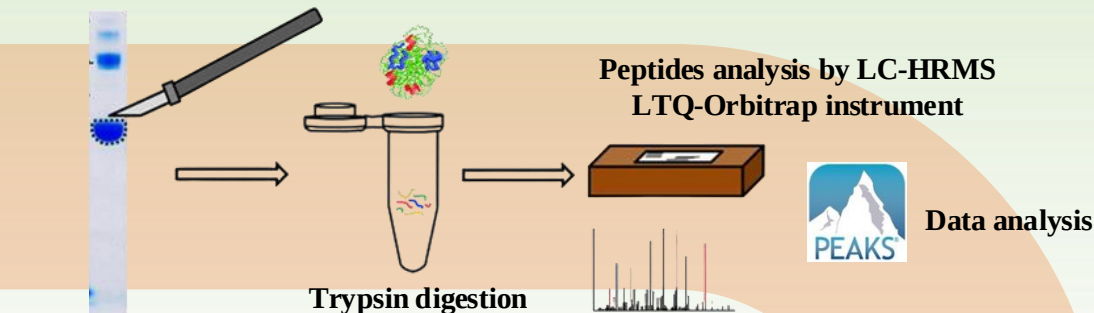
Protein extracts



SDS-PAGE



Protein identification



In silico approach

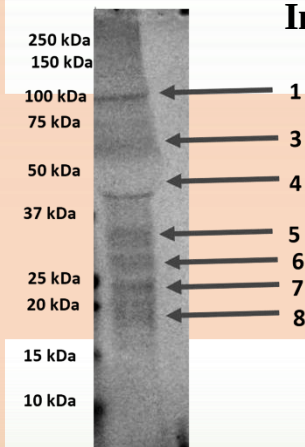


HR-MS coupled with *in silico* is efficient for assessment of the allergenic potential of proteins

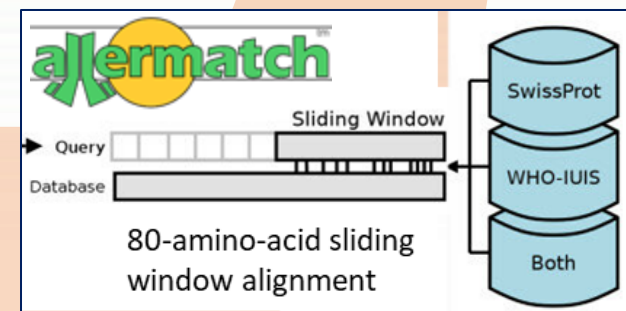
Immunoblotting assays with sera of patients with positive specific IgE to known legume allergens, demonstrated cross-reactivity with other sources of proteins

In vitro approach

Immunoblotting IgE specific
with human sera

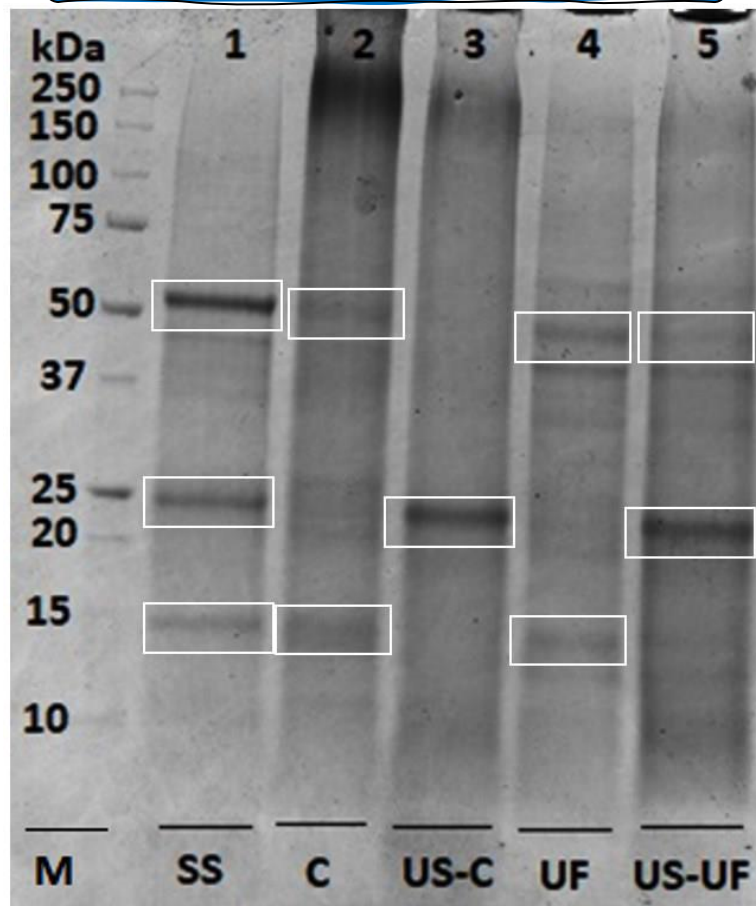


Patients allergic
to legumes



Sequence alignment with known allergens

SDS-PAGE analysis



TOTAL PROTEIN CONTENT (%)

Sample	AAs sum	General CF: 6.25	Estimated CF: 5.8 *
SS	23.3 ± 0.86 ^c	39.83 ± 0.18 ^a	36.96 ± 0.12 ^b
C	51.0 ± 0.58 ^c	69.55 ± 0.79 ^a	64.54 ± 0.52 ^b
US-C	41.1 ± 0.63 ^c	52.36 ± 0.05 ^a	48.59 ± 0.03 ^b
UF	42.2 ± 1.42 ^c	58.82 ± 0.81 ^a	54.59 ± 0.53 ^b
US-UF	42.0 ± 0.50 ^c	54.86 ± 1.07 ^a	50.91 ± 0.70 ^b

* Actual conversion factor
(from total amino acids content)

Large subunits of RuBisCo (56 kDa)

Ribulose-1,5-bisphosphate carboxylase/oxygenase

Chlorophyll a-b binding protein (25 kDa)

Small subunits of RuBisCo (14 kDa)

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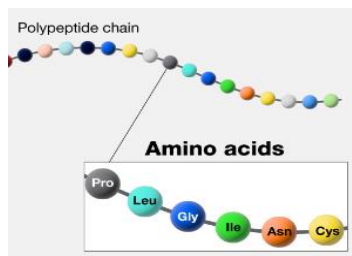
IN SILICO ANALYSIS



Preliminary insight
on homologies with other
known allergens

Limited information on
protein existence of some
Lemna gibba proteins

Protein AAs
sequence



Cross reactivity:
sequence homology
**> 35 % over 80 AAs
window ⁽⁷⁾.**

Sequence homology **> 70 %:**
high probability of
cross-reactivity

Sequence homology **< 50 %:**
rare cross-reactivity ⁽⁸⁾

Possible cross-reactivity
with **spinach and celery**

Need of further studies with
***in vitro* immunoblotting assays**

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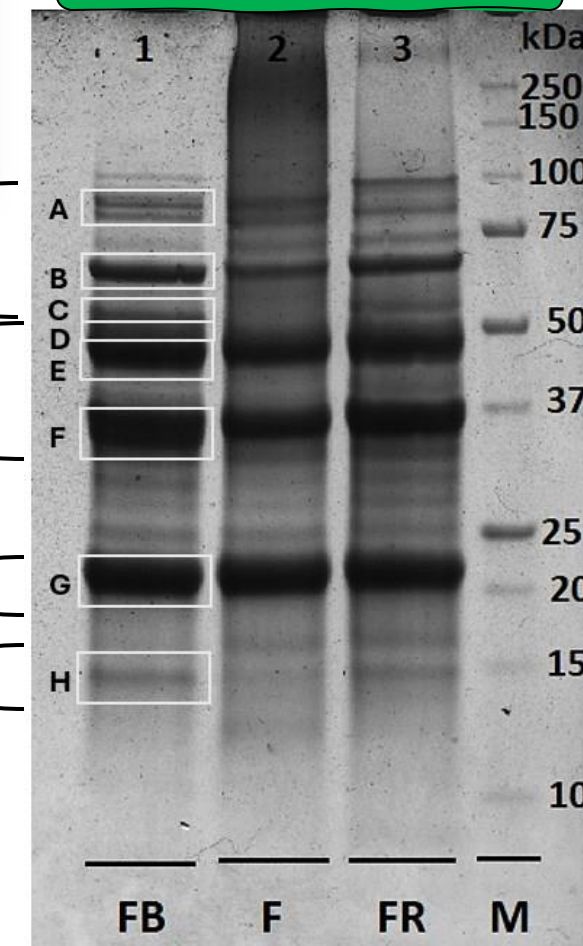
TOTAL PROTEIN CONTENT (%)

Sample	AAs sum	Literature CF: 5.4 ⁽¹⁰⁾	Estimated CF: 5.5 *
F	65.7 ± 0.49 ^a	64.6 ± 0.10 ^b	65.8 ± 0.10 ^a
FB	75.5 ± 1.13 ^a	73.2 ± 0.28 ^b	74.6 ± 0.29 ^{ab}
FR	n.a.	73.1 ± 0.68	74.4 ± 0.69 [*]

* Actual conversion factor (from total amino acids content)



SDS-PAGE analysis



FB: bleached faba bean extract (lane 1); F: faba bean extract (lane 2); FR: reference faba bean extract (lane 3); M: markers (kDa)

Probable **cross-reactivity** with other legumes (higher with **pea**, **lentil**, **soy**)

In vitro immunoblotting assay

A, B, C: **Convicilin** (95, 73, 55 kDa)

D, E, F: **Vicilin** (50, 49, 35 kDa)

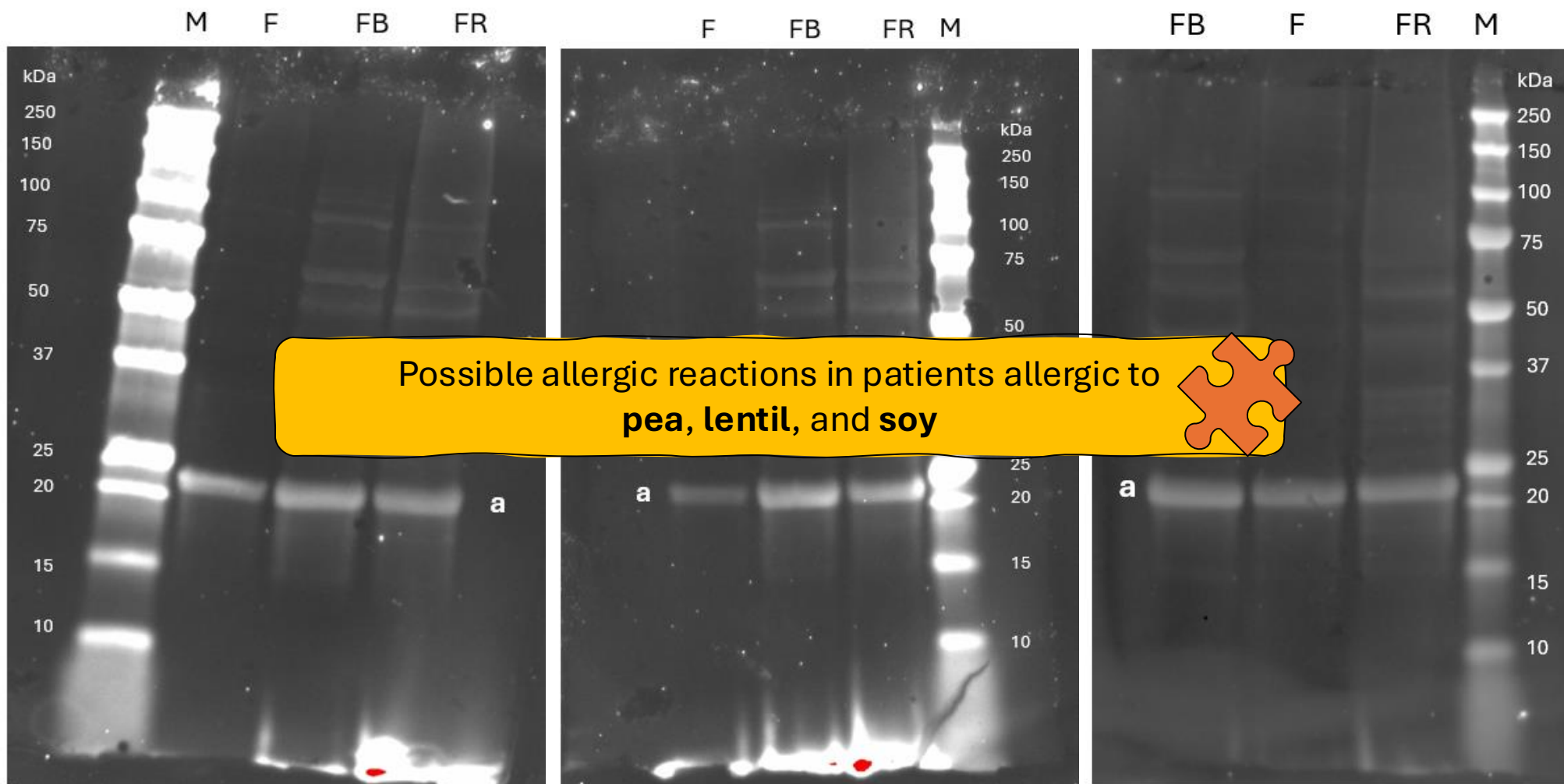
G: **Beta chain of legumin type B** (22 kDa)

H: **Albumin** (15 kDa)



IN VITRO IMMUNOBLOTTING ASSAY

Most intense
Legumin B beta chain
(22 kDa, band a)
in all samples



Pea allergy

Soy allergy

Lentil allergy

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TOTAL PROTEIN CONTENT (%) $\pm$ SD

Sample	AAs sum	Lit. CF: 5.84 ⁽¹²⁾	Est. CF: 5.59
T	36.0 $\pm$ 1.25 ^c	40.8 $\pm$ 0.16 ^a	39.1 $\pm$ 0.15 ^b



SDS-PAGE
(reducing conditions,
50 μ g of protein)

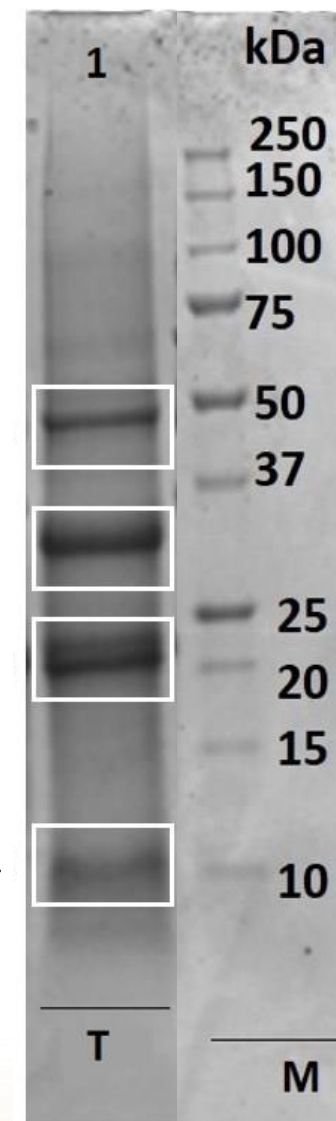
Proteins **mainly**
from seeds
PG2 and ns-LTP
high in peels

Polygalacturonase-2 (PG2) (49 kDa)

Pectinase (PE) (35 kDa)

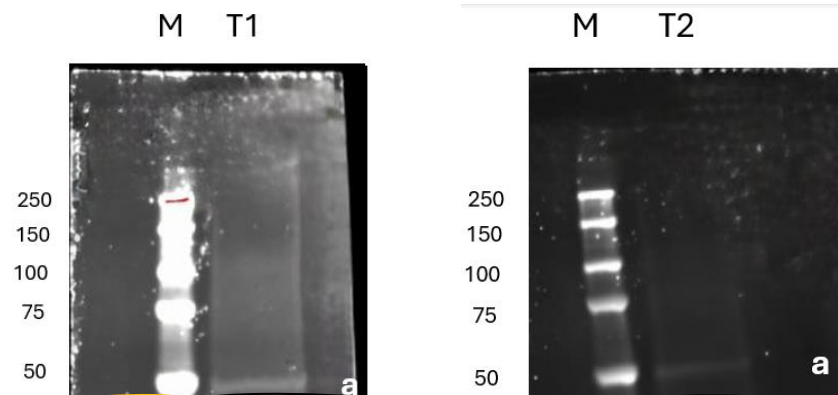
Acid beta-fructofuranosidase (β FRU) (20 kDa)

Non-specific lipid-transfer protein (ns-LTP) (9 kDa)



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Possible variations in protein content and allergens profile based on type of product (seeds, pulp and peel ratio)

Crude tomato allergy

Cooked tomato allergy

IN VITRO IMMUNOBLOTTING ASSAY

Tomato allergens
well characterized on



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Sola l PG *
allergen

Sola l 2
allergen

Most reactive
Polygalacturonase-2 (PG2)
(49 kDa, band a)

Acid beta-fructofuranosidase (βFRU)
(20 kDa, band c)

* Not in WHO-IUIS Official
Nomenclature List

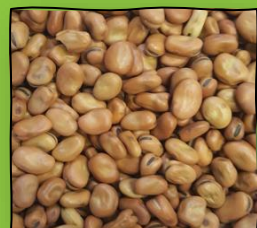
Immunoreactivity
towards **both sera**

ALLERGENICITY POTENTIAL ASSESSMENT



Tailored
to each protein matrix

Significant role of **level of knowledge about specific proteins** in the assessment process



Further studies on:

Possible impacts of **applied treatments** in
modulating the **allergenicity**



Potential cross-reactivity with
other known **allergens**

Future perspective → Protein identification with mass spectrometry



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**Sara Cutroneo
Luisa Calcinai
Barbara Prandi**

**Thank you all
for your kind attention**



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