EFFECT OF TECHNOLOGICAL TREATMENTS ON IMMUNOREACTIVITY AND ALLERGENICITY OF THE ALLERGENIC PROTEIN PRU P 3 FROM PEACH

Tobajas, A.P.[1], Catalán T, Segura-Gil I, Sánchez, L.[1], Colás, C.[2], Calvo, M.[1], Pérez, M.D.[1*]

(1) Departamento de Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Instituto Agroalimentario de Aragón (IA2) (Universidad de Zaragoza-CITA), Zaragoza, España.

*(2) Servicio de Alergología. IIS-Aragón. Zaragoza, España. * dperez@unizar.es*

Facultad de Veterinaria Universidad Zaragoza

Instituto Universitario de Investigación Mixto Agroalimentario de Aragón Universidad Zaragoza

INTRODUCTION

In the Mediterranean area, about 70% of cases of allergy to fruits are associated with the consumption of fruits from the Rosaceae family, being peach the fruit which most often cause allergies. Pru p 3 is the most allergenic protein of peach, as more than 90% of allergic individuals to this fruit have IgE to it. Pru p 3 shows a high resistance to heat and digestive proteolysis giving it the capacity to produce allergic reactions of considerable severity, like anaphylactic shock. Since peach is often consumed processed in the form of juice, nectar, jam, etc, it is of great interest to study if technological treatments that could be applied in the preparation of such products may reduce its potential allergenicity.

The aim of this work was to study the effect of different technological treatments on the degradation and potential allergenicity of Pru p 3 which could be applied in fruit processing industry.

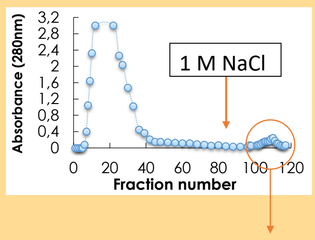
EXPERIMENTAL

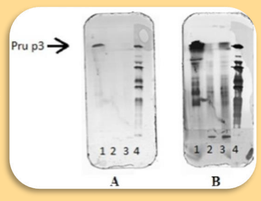
Isolation of Pru p 3 protein from fresh peach extract

Extraction with phosphate buffer pH 6,0 containing 2mM EDTA, 10 mM DIECA, 2% PVPP

SDS-Electrophoresis

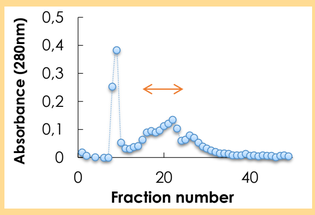
Cationic exchange chromatography SP-Sepharose

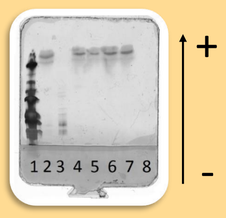




(1) Peak 2 (concentrated)
(2) Peak 1
(3) Peach extract
(4) MW Marker
A) Coomassie Blue Staining
B) Silver Staining

Gel filtration chromatography Sephadex G-50





(1) MW Marker
(2) Peak 2 of SP-Sepharose
(3) Fraction 9
(4) Fracción 16
(5) Fraction 18
(6) Fraction 20
(7) Fraction 22
(8) Fraction 26

Mass spectrometry (MALDI-TOF) confirmed Pru p3 as the isolated protein

Sample	identified protein	MW (Da)	Number of peptides matched	sequence coverage (%)	Fragmented Peptides (MH+, sequence)
Purifed Peach Protein	gi|17974195 Pru 1	9594	8	92	1534.72, TTPDRQAACNCLK 1905.00, QLSASVPGVNPNNAAALPGK 2008.00, ITCGQVSSSLAPCIPYV
	gi|83754020 Pru p3	9725	8	91	1534.72, TTPDRQAACNCLK 1905.00, QLSAAVPGVNPNNAAALPGK 2008.00, ITCGQVSSSLAPCIPYV

Obtention and characterization of antisera

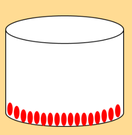
Inoculation of Pru p 3 in rabbits

Antisera anti-Pru p 3

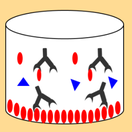
→  → **Purification of antibodies** → **Conjugation of antibodies with peroxidase** → **ELISA sandwich**

Competitive ELISA

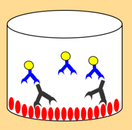
Allergenicity



Coating wells with Pru p 3



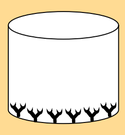
Incubation with human serum of peach allergic patients



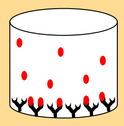
Incubation with fluorescence labelled human anti-IgE

Sandwich ELISA

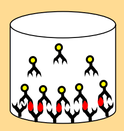
Antigenicity



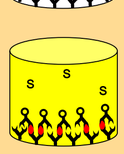
Coating wells with antibodies anti-Pru p 3



Incubation with standard or sample



Incubation with peroxidase conjugated IgG anti-Pru p 3



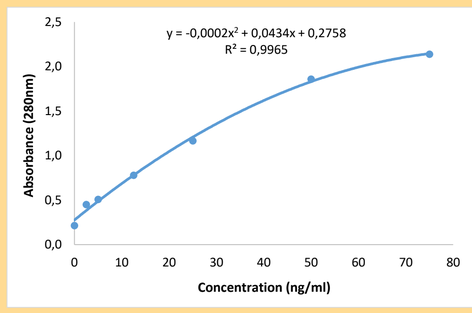
Incubation with substrate

Pru p 3 protein | Human IgE anti-Pru p 3 | Human anti-IgE fluorescenced labelled

Pru p 3 protein | Rabbit IgG anti-Pru p3 | Peroxidase conjugated IgG anti-Pru p 3 | S Substrate

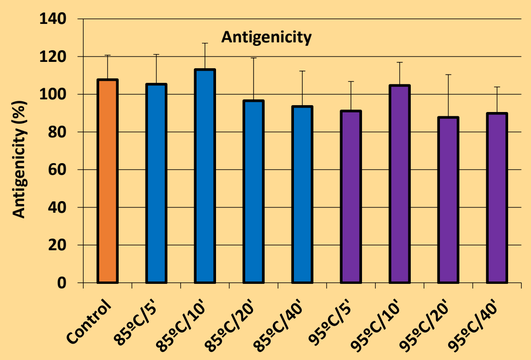
RESULTS

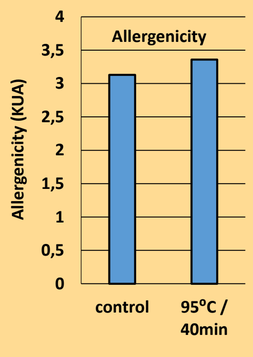
Calibration curve for the determination of Pru p 3 by the sandwich ELISA



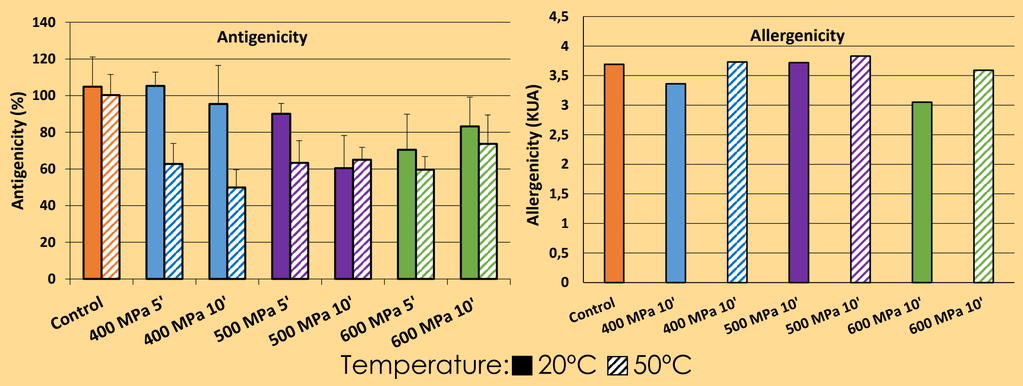
Linear range: 2.5-75 ng/mL
Limit of detection: 1 ng/mL
Limit of quantification: 9 ng/mL

Effect of heat treatment on denaturation of Pru p 3

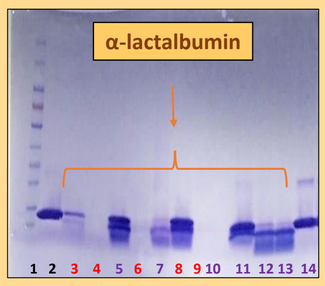


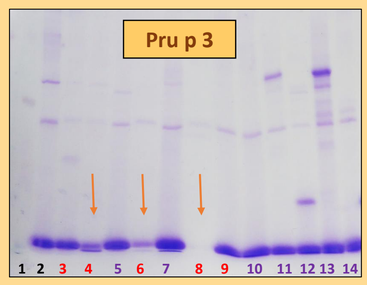


Effect of high hydrostatic pressure on denaturation of Pru p 3



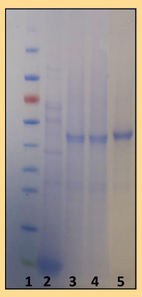
Effect of different proteases (n = 20) on the degradation of Pru p 3 protein



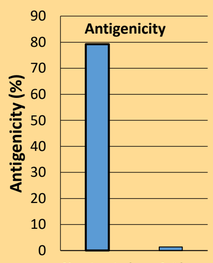


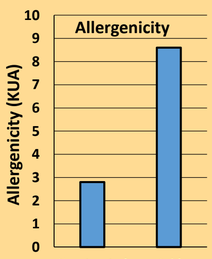
1. Molecular weight marker
2. Non-treated protein
3 , 4, 6, 8, 9. Treated with alkaline protease
5, 7, 10, 11, 12, 13, 14. Treated with acid proteases

Most proteases assayed did not degrade Pru p 3 whereas they had a marked proteolytic effect on α-lactalbumin.



1. Molecular weight marker
2. Non-treated Pru p 3
3 , 4. Tretaed Pru p 3
5. Acid protease





Only one acidic protease was able to degrade Pru p 3 and decrese its antigenicity and allergenicity

CONCLUSIONS

- **Heat treatments applied to peach extract decreased the antigenicity of Pru p 3 (IgG), but they did not affect its allergenicity (IgE).**
- **High hydrostatic pressures treatments applied to peach extract did affect the antigenicity but not the allergenicity of the Pru p 3.**
- **Of all proteases assayed, only an acid and two alkaline proteases were able to degrade Pru p 3 efficiently at their optimal conditions.**
- **Only experimental conditions in which the acid protease is effective, could be used in the peach juice industry . These results are promising as they could be applied to manufacture hypoallergenic juices .**