Fellowship type: EAACI Medium-term Fellowship (6 month period)

Applicant: África Sanchiz Giraldo, M.S.

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Project: Study of the allergenic properties of tree nuts subjected to thermal processing and identification of potential new allergens

Host Supervisor: Univ.-Prof. Dr. med. Natalija Novak

Host Institution: Department of Dermatology and Allergy, University of Bonn Medical Center, Sigmund-Freud-Str., 25, 53127 Bonn, Germany

With the cooperation of: Dr. rer. nat. Beatriz Cabanillas

Dates of Fellowship: 1 April 2017 – 30 September 2017
Dear EAACI headquarters,

First of all, I would like to thank EAACI for the opportunity to carry out this experience of six months in the Uniklinikum Bonn - Medical Center, in the Prof. Dr. med Novak group within the Dermatology and Allergy Department.

During this research stay we have addressed different questions following the plan previously established to achieve the defined objectives.

- Analysis of the effects of thermal processing on the allergenicity of tree nuts such as cashew and pistachio
- Analysis of the impact of thermal processing on the digestibility of tree nuts allergens
- Analysis the IgE-binding protein profiles from tree nuts as walnut and chestnut using sera from Spanish patients with walnut or chestnut allergy

What is the influence of thermal treatments on the allergenicity of cashew and pistachio nuts?

Food processing can be used as a tool to modify different properties in foods, as allergenicity, among others. In this case, six different boiling and autoclave treatments (different time, pressure and temperature) were applied on pistachio and cashew nuts to determine the effect on their IgE reactivity, using sera from Spanish allergic patients.

During these months in Uniklinikum Bonn-Medical Center, Dermatology and Allergy Department, traditional immunoassays have been performed using sera from Spanish allergic patients to cashew and pistachio, in order to determine alteration of the allergenic properties of processed nuts: IgE ELISA, inhibition ELISA and western blot. Detection of allergenic proteins using specific antibodies has been also studied.

Additionally, physiologically relevant assays based on mediator release assay using rat basophil leukemia cell line and skin prick test (SPT) have been carried out.

As main results, we can conclude that moist thermal processing diminished the IgE-binding properties of pistachio and cashew compared to control, especially after autoclave treatment. The wheal size in SPT was importantly reduced after application of thermally-treated samples and degranulation of basophils after the challenge with pistachio and cashew treated samples was importantly diminished compared to untreated ones. We observed that detectability of specific proteins in cashew/pistachio was also affected by heat/pressure treatments. We have initially
determined by LC-MS/MS that some peptides from major allergens of cashew and pistachio nuts are potentially resistant to harsh conditions of autoclave.

Further studies would be necessary to analyze the decreased IgE cross-linking capacity of such samples with *in vivo* models of food allergy. In the future, these treatments might be useful for immunotherapeutic approaches in pistachio and cashew allergic patients.

*What is the impact of thermal treatments on the enzymatic digestibility of protein extracts from treated and untreated cashew and pistachio?*

Enzymatic digestion with pepsin and trypsin was applied in cashew and pistachio protein extracts from untreated, boiled and autoclaved samples, simulating gastric and duodenal in vitro conditions. By monodimensional electrophoresis, we observed that cashew protein extract (treated and untreated) seemed to be more resistant to pepsin/trypsin digestion than pistachio extracts at the same conditions, although in both tree nuts there are several proteins highly resistant to 90 minutes of pepsin digestion and overnight trypsin digestion.

*Which is the nature of potential new allergens in chestnut?*

We have analyzed the IgE binding protein profiles by western blot using sera from Spanish allergic patient to chestnut, after separation by SDS-PAGE. We have performed a preliminary study by mass spectrometry to determine the nature or identification of some of these potential new chestnut allergens, not described as food allergens yet.

**Acknowledges and personal reflection**

I would like to kindly thank my hosts in the Uniklinikum Bonn, Prof. Dr. med Natalija Novak and Dr. Beatriz Cabanillas, for their continuous supervision and support during these months. They have provided me their time and knowledge in any moment, making possible the realization of most of the experiments previously stablished in the EAACI fellowship project. I extent my gratitude to my PhD supervisors, Dr. Cuadrado and Dr. Linacero, for their constant encouragement during this research stay.

Prof. Dr. med Novak's laboratory counts with all the installations needed to develop the planned experiments, and a very competitive and homely research group composed by high quality researchers and very well trained technicians. On the other hand, Uniklinikum Bonn also counts
with high quality core facilities. In conclusion, I strongly recommend the center and the department for future research scientists.

I would like also thank the European Academy of Allergy and Clinical Immunology (EAACI) all the assistance during my Fellowship. The EAACI fellowship exceeded expectations and it has served to consolidate the collaboration initiated time ago between the home and the host research group. This research experience has been beneficial for my PhD project and it allows maintaining the fruitful collaboration between the home and the host institutions in the future. The received amount is very generous and enough to fund travel and living costs in any European country. Establishment of minimum and maximum spending limits within the total amount of money would be recommended (for living/travel and laboratory expenses).

A publication from this work in a journal with high Impact Factor is planned for next months.