Isolation and characterisation of 2S albumins: developing clinical tools for allergy diagnosis

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Background: 2S albumins have been described as food allergens constituting important clinical diagnostic tools in food allergies. Despite their relatively low sequence similarity, there are studies focused to their potential role in cross-reactivity reactions, so they can be used as a tool for their prediction.

Aims: Identification of ten 2S albumins from seeds and nuts, characterization of its structural parameters and assayed them for IgE reactivity with patients’ sera.

Methods: Isolation of 2S albumins from extracts using chromatographic methods. After identification by mass-spectrometry, molecular characterization was conducted by electrophoretic methods (1- and 2-dimensional electrophoresis); its secondary structure and thermal stability were studied by circular dichroism spectroscopy. Finally, immunoassays were performed to reveal their allergenic capacity, using allergic mustard-allergic patients’ sera.

Results: Proteins have been isolated and purified from extracts by means of two chromatographic steps, a gel filtration and a reverse phase in HPLC. They were identified as 2S albumins by mass-spectrometry of digested peptides (MALDI-TOF). They generally show a helicoidal secondary structure, stable at 85ºC and possess a wide range of pi and heterogeneous polypeptide size and composition.

Discussion: The fingerprint analysis confirmed the purified proteins as 2S albumins, storage proteins already characterized as allergens. Purified proteins shown similar characteristics than those described for this family of proteins. The molecular characterization revealed low molecular masses and acid and basic pi, some of them exhibit the typical two chains-pattern and others, such as the one from sunflower seed, are constituted by one polypetide chain. Circular dichroism studies reveal their high resistance to thermal treatment except the one from pistachio.

Conclusions: The characterized 2S albumins will be used as clinical tools in Component-Resolved Diagnosis (CRD) in a big Spanish population of patients allergic to vegetable foods using the potent high-throughput screening technology with ADVIA-Centaurus, to elaborate a more accurate diagnosis and therefore a more effective treatment. This diagnosis is especially relevant having in account the severe symptoms caused by this allergenic family.