THESIS SUMMARY

Food allergy is one of the frequent disorders and its incidence in paediatric as well as adult population is continuously rising, having doubled in the last two decades. Although wheat belongs to major food allergens and is a staple food in most diets, we have only little knowledge of wheat proteins causing IgE mediated hypersensitivity reaction. Diagnostic approaches of food allergy to wheat have a high sensitivity, but low specificity. Poor predictability and specificity may be associated with the insufficient purity of wheat extracts used in sIgE assays or with the lack of major allergens in these extracts.

In the first step, we characterized 19 potential allergens recognised by IgE Abs of allergic patients, using proteomic techniques (1-DE, 2-DE, MALDI-TOF, QTOF and LCQ^{DECA} nLC-MS/MS ion trap technique). We identified these IgE-binding molecules such as: α-amylase inhibitors, β-amylase, profilin, serpin, β-D-glucan exohydrolase and 27K protein. To quantify sIgE in patient's sera we developed ELISA using the whole wheat extract and two commercially available α-amylase inhibitors.

Second, we developed a procedure that allows isolation of wheat allergens from natural sources using Rotofor cell and HPLC. Twenty-seven potential wheat allergens have been successfully identified; of these, the following seven are newly reported in food allergy: endogenous α-amylase/subtilisin inhibitor, trypsin/α-amylase inhibitor CMX1/CMX3, TLP, XIP, β-glucosidase, class II chitinase and 26 kDa endochitinase. The biological activity of purified allergens was tested using the basophil activation test. We have shown for the first time that purified allergens, such as α-amylase inhibitor 0.19, lipid transfer protein, TLP and wheatwin, can activate patients' basophils, confirming that our purified proteins maintain their biological activity.

Third, we investigated how thermal processing influences the ability of OVA to induce allergic symptoms and immune responses in mouse model of food allergy.

The aim of our studies is to identify the most important wheat allergens in IgE mediated hypersensitivity reaction. We developed new procedures of identification and isolation of allergens in their native form in amounts sufficient both for biological testing (*in vivo* and *in vitro*) and for physicochemical characterization. Such studies will lead to a more detailed knowledge of allergenicity of wheat proteins and to improved specificity of diagnostic tests.