ABSTRACT

The great interest of the research activity in food allergies could be attributed to the increase of allergic reactions all over the world not only in infants but even in adult age.

As an alternative to the development of an allergen-free diet, many works have been focused on a novel approach for the treatment of allergens: instead of eliminating the allergens from the diet, the immunoresponse can be reduced or even eliminated by inducing some modifications of their molecular structure. In fact, changes in allergen conformation can modulate its identification by the specific antibody produced by immune system in allergic reactions.

Structural modifications in allergens could be induced by conventional thermal treatments as well as by non-thermal technologies, namely High Hydrostatic Pressure (HHP), Pulsed Electric Fields (PEF), Pulsed Light (PL) and γ-radiations.

Non-thermal technologies have been widely used in the last years for food preservation, having the advantage of increasing the shelf-life and freshness of the raw food products. These technologies are able to affect the food nutritional and organoleptic properties only slightly thanks to the use of a non-thermal stress to treat foodstuffs. Among them High Hydrostatic Pressure technology has been successfully used in food pasteurization, but also in processes involving the sol-gel transition such as the production of jams, jellies and dairy products. The ability of High Pressure to determine structural changes in foods was studied in order to assess if proteins unfolding and/or aggregation and gelation can be induced and if the treatment affects the functional properties and digestibility of proteins. These effects were studied on particular proteins, namely the allergens, for which unfolding and structural modification have been proven. However, the effectiveness of the High Pressure processing on the reduction of immunoresponse reduction was not clearly assessed so far.

The objective of this PhD thesis was the study of the modifications induced by High Pressure Process on allergenic proteins and the possibility of obtaining hypoallergenic peptides by means of a combined High Hydrostatic Pressure hydrolysis. In particular, the effect of the HHP on the allergens structural modification was investigated in a wide range of operating conditions, including both gelling and ungelling conditions. Rheological behavior and functional properties of HHP processed allergens was also determined.

After having identified the product characteristics and the operating conditions at which the physical transition from unfolding to aggregation takes place, also the effects of HHP on hydrolysis reaction by proteolytic enzymes were analyzed.

Chemical-physical properties and allergenicity of peptides obtained by HHP were determined. For the sake of comparison, also the characteristics of the peptides produced at atmospherical pressure were measured.

Finally to quantify the effect of the optimized process on the allergenicity of HHP peptides, their immunoresponse was analyzed by *in vivo* tests.

The results of the experimental activity have demonstrated that:

a) High Hydrostatic Pressure is a technology able to induce protein modifications whose extent depends on processing conditions and product characteristics. In gelling proteins, such as Bovine Serum Albumin (BSA), operating conditions namely pressure level, temperature and holding time as well as product characteristics, namely protein concentration and isoelectric point, can affect the unfolding/gelling transition.
b) The rheological behavior of HHP induced allergen gels depends on the treatment conditions, which determine gel hardness/consistence. Furthermore, higher deviations of the gels from Newtonian behavior can be observed if higher pressure levels and holding time are applied.
c) At pressure levels lower than that corresponding to the unfolding/gelling transition is also possible to detect the effect of high pressure on allergens at the microscopic scale. At these conditions, the application of high hydrostatic pressure induces the anticipation of thermal denaturation by reducing the sol/gel transition temperature.
d) High Hydrostatic Pressure causes conformational changes and the unfolding of allergens, as demonstrated by the increase in foaming properties and the exposure of thiols reactive groups. These effects are likely to occur up to certain values of pressure and holding time and up to certain values of protein concentration while in more drastical treatment conditions and with higher protein concentrations, the aggregation replaces the unfolding.
e) If the hydrolysis is assisted or anticipated by High Hydrostatic Pressure treatments carried out at the operative conditions maximizing protein unfolding, higher hydrolysis degree are detected and the peptides show a lower size. At very high pressure, due to the formation of protein aggregates and the reduction of enzymatic activity, the hydrolysis reaction rate is drastically reduced.
f) High Hydrostatic Pressure treatment can be applied also to non-globular proteins, such as caseins. Also with this protein, hydrolysis

reaction is enhanced and lower dimension and more homogenous peptides mixture can be produced.

g) The kinetics of protein hydrolysis by proteolytic enzymes is faster under high hydrostatic pressure then at atmospheric conditions, as shown by the increase in the reaction constant.

h) Peptides produced by hydrolysis assisted or induced by High Hydrostatic Pressure can be considered as hypoallergenic compounds being their immunoresponse against antibodies reduced as shown by in vivo tests (*Prick tests*).