Summary

Mucosal passive immunization is the transfer of active antibodies from one organism to the mucosal surfaces of another organism for preventing or treating infectious diseases. Mucosal passive immunization has a great potential for the prevention and treatment of enteric infections like Rotavirus, which causes more than 114 million episodes of diarrhoea annually with a death toll of more than 450.000 per year. However, the high cost of recombinant antibodies with the current manufacturing systems based on mammalian cells hampers the production of the high antibody quantities required for passive immunization strategies. Alternative expression platforms such as plants could provide higher scalability and reduced costs. Moreover, the use of edible plant organs, which are *Generally-Regarded-As-Safe* (GRAS), could reduce manufacturing costs even further by easing the requirements for antibody purification. We analyze here the feasibility of utilizing fruits as inexpensive biofactories of human antibodies that can be orally delivered as crude extracts or partially purified formulations in mucosal passive immunization strategies.

In the first section of this thesis, the construction of tomato plants producing a model human Immunoglobulin A (IgA) against rotavirus in their fruits is described. As a result, an elite homozygous line was obtained whose fruits produced on average 41 μg of IgA per gram of fresh weigh, equivalent to 0.69 mg IgA per gram of dry tomato powder. Minimally processed products derived from IgA-expressing tomatoes were shown to strongly inhibit virus infection in an *in vitro* neutralization assay. Moreover, in order to make IgA-expressing tomatoes easily distinguishable from wild-type tomatoes, they were sexually crossed with a transgenic tomato line expressing the genes encoding *Antirrhinum majus* Rosea1 and Delila transcription factors, which confer purple colour to the fruit. The resulting transgenically-labelled purple tomatoes contained not only high levels of recombinant neutralizing human IgA but also increased amounts of anthocyanins.

In the second section of the thesis the composition of IgA-expressing tomatoes was analyzed in search of possible unintended effects that could compromise the GRAS status of the final product. To this end, transgenic IgA-tomatoes were compared with wild type tomatoes and also commercial tomato varieties using proteomic and metabolomic approaches. 2D-DIGE gels coupled with LC-MSMS for protein identification showed that all the uptrend differential proteins detected corresponded only to immunoglobulin chains or antibody fragments. On the other hand, non-targeted metabolite data obtained by UPLC-MS

identified variations between transgenic and non-transgenic lines, however such variations could not be associated with the presence of abnormal levels of any particular secondary metabolite in the IgA fruits. Therefore from the analysis conducted here no sign was obtained that could indicate that tomato-IgA formulations are less safe for consumption than their wild type counterparts.

The third section of this thesis focused in optimizing the production of the secretory form of the IgA (sIgA), as this is the most convenient antibody isotype for mucosal passive immunization. SIgA production requires the co-expression of four transcriptional units encoding the light chain (LC), heavy chain (HC), joining chain (JC) and secretory component (SC). In order to optimize its production, sixteen versions of a human sIgA against rotavirus comprising different antibody chain isotypes with or without retention in the endoplasmic reticulum were constructed using the GoldenBraid multigene assembly system. Transient expression in *Nicotiana benthamiana* of all sIgA versions showed that maximum expression levels were achieved by the sIgA version containing alpha1 HC, lambda LC and with a KDEL signal linked to the SC, with an estimated 33% of the total IgA accumulating in the form of a secretory complex.