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## **Antibodies Collection dedicated to food components**

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Due to their high selectivity, antibodies are useful tools to detect, quantify or characterise proteins or peptides from complex samples. Numerous antibodies against wheat, milk and egg components were obtained at INRA. This large library of antibodies enables the diversity of composition of raw material and impact of processes and digestion to be addressed.

## Raw material

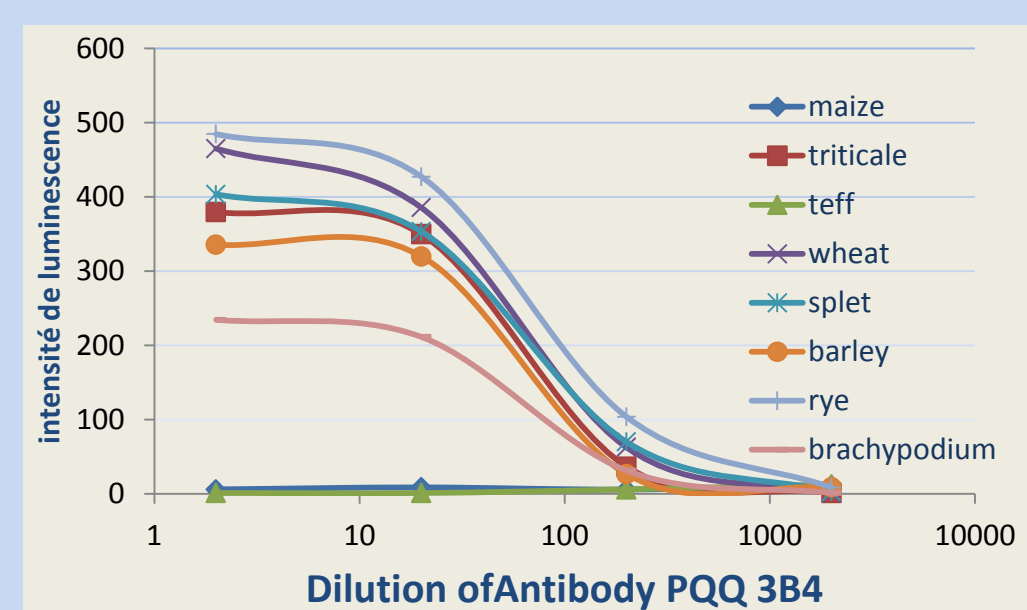
### Cereals

Antibodies against wheat grain proteins i.e. proteins from gliadin, glutenin and albumin/globulin fractions have been produced from purified proteins or synthetic peptides.

Immunogene	Clone	purified prolamins					
		α-gliadins	β-gliadins	γ-gliadins	ω 1,2-gliadins	α-gliadins	LWM Glutenins
C-terminal or N-terminal	GLIA 2E5						
	S3 B512						
	ONT 18A51D2						
repetitive domain	F98 6x1						
	S1 DG35						
	S1 EG7D11						
	S1 DH3						
	PQ3 3B4						

Antibodies have been fully characterised by ELISA with highly purified prolamins proteins. Some of these antibodies enable gliadins or glutenins subclasses to be distinguished from one another, others are suitable for the detection of the whole gliadin or glutenin fractions or whole prolamins.

Combining Macro-Array spotting with chemiluminescent ELISA, the Wheat Antibody Collection was screened against « prolamin-like » proteins from several cereals.



Most of the antibodies bound proteins from spelt, rye, barley and triticale, reflecting the genetic closeness of these cereals. Interestingly some of them also reacted on brachypodium, the new model plant for temperate cereal.

### Egg

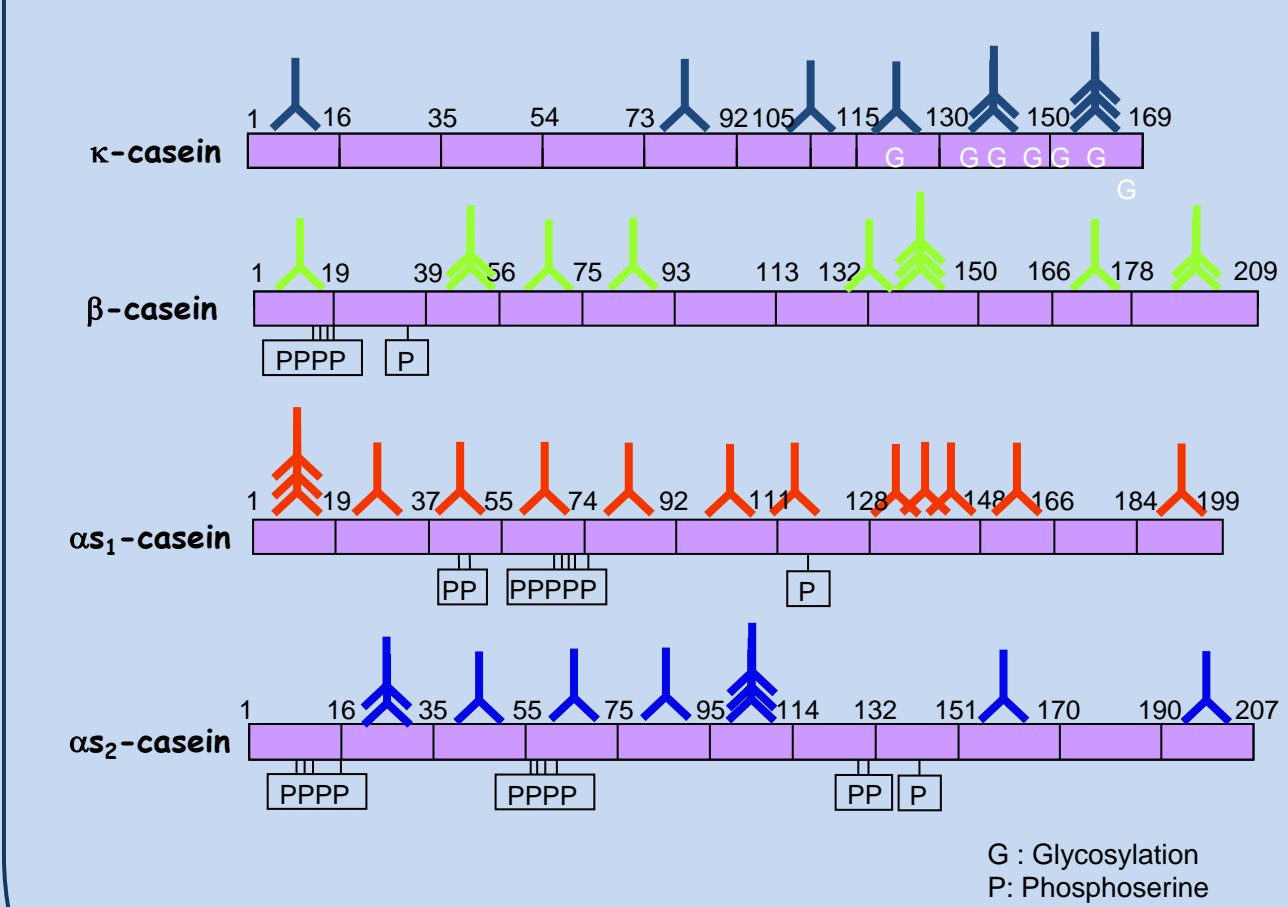
Numerous monoclonal antibodies against purified proteins from hen's egg have been produced. Antibodies specific of ovalbumin, ovomucoid, ovotransferrin and lysozyme from the white and phosvitin, LDL and HDL from the yolk have been fully characterised and are now available for characterisation studies of raw material.

	Grp 1 9 Mabs	Grp 2 4 Mabs	Grp 3 2 Mabs	Grp 4 4 Mabs	Grp 5 3 Mabs	Grp 6 1 Mab	Grp 7 1 Mab	Grp 8 1 Mab
Hen	+	+	+	+	+	+	+	+
Duck	-	-	-	-	-	+	+	+
Turkey	-	-	+	+	-	+	+	+
Guinea Fowl	-	+	-	-	-	+	+	+
Quail	-	-	-	+	+	-	+	+

Their cross reactivity towards egg's proteins from other species have been evaluated. Depending on the Mab, some of them enable the detection of duck, turkey, guinea fowl or quail.

### Milk

In order to develop immunoassays, INRA produced a collection of polyclonal and monoclonal antibodies specific of endogenous components of milk : caseins, α-lactalbumin, β-lactoglobulin, cathepsin D, plasmin/plasminogen lactoferrin, and of cheese manufacturing auxiliaries. These probes have been characterised against purified proteins and peptides.



Several monoclonal antibodies directed against β, κ, α<sub>1</sub> and α<sub>2</sub> caseins were produced. Specificities were determined using libraries of synthetic peptides and peptides fractionated from tryptic hydrolysis of purified caseins.

Johansson et al. 2009, Mol. Immunol. 46, 1058-1066

## Process

### Wheat deamidation

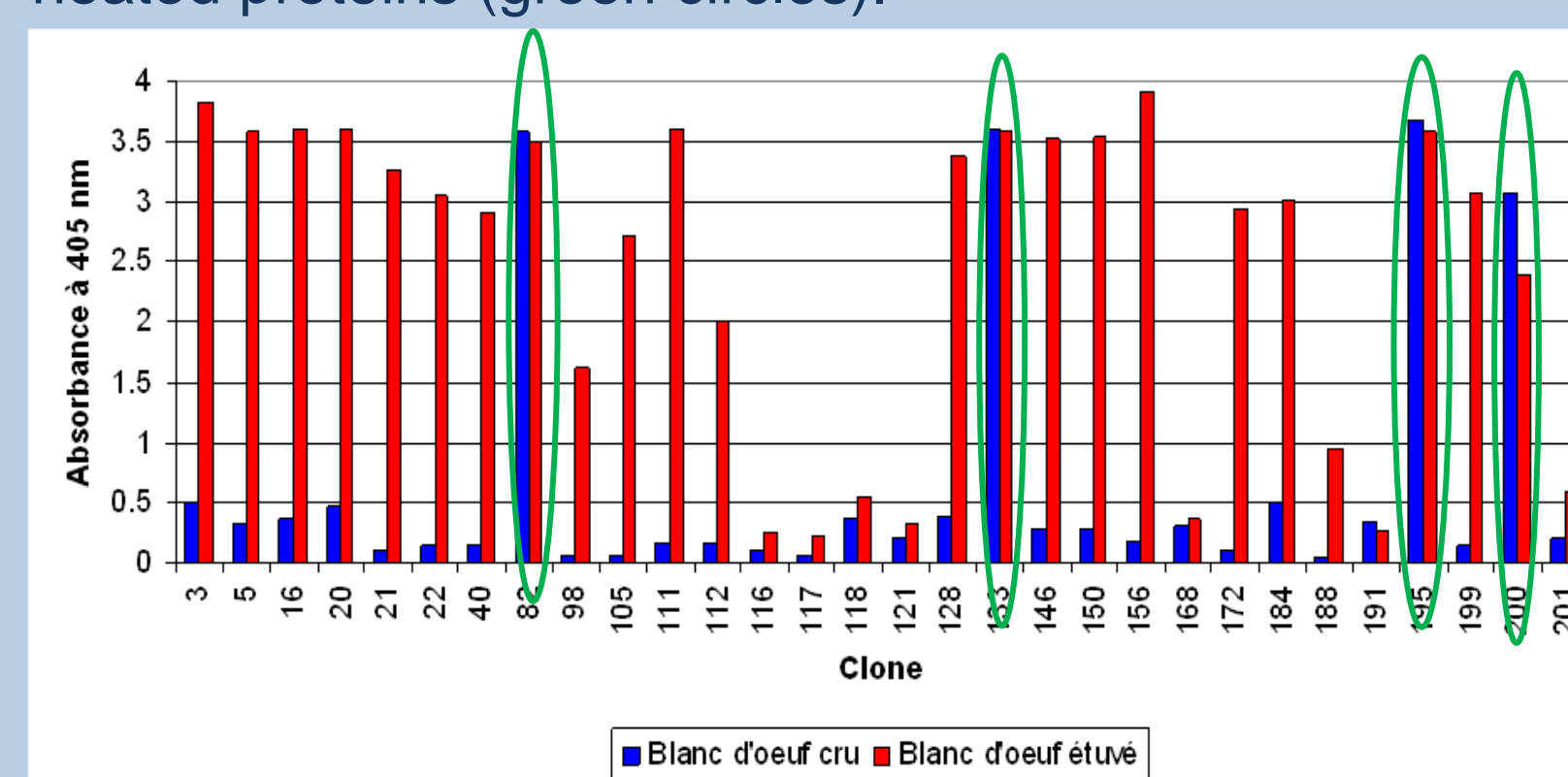
In some food products, gluten is added after partial hydrolysis or deamidation in order to enhance their use as functional ingredients. These processes lead to the replacement of some glutamine by glutamic acid which may impair the immunochemical detection of the wheat prolamins.

Within the collection some antibodies capable of detecting these modified proteins have been identified. They are useful tools to monitor modified glutens

Origin of the immunogenic peptide	Clone	deamidated proteins				
		α-gliadins	β-gliadins	ω 1,2-gliadins	α-gliadins	β-gliadins
C-terminal or N-terminal domain	GLIA 2E5					
	S3 B512					
	ONT 18A51D2					
repetitive domain	F98 6x1					
	S1 DG35					
	PQ3 3B4					
	S1 EG7D11					
	S1 DH3					

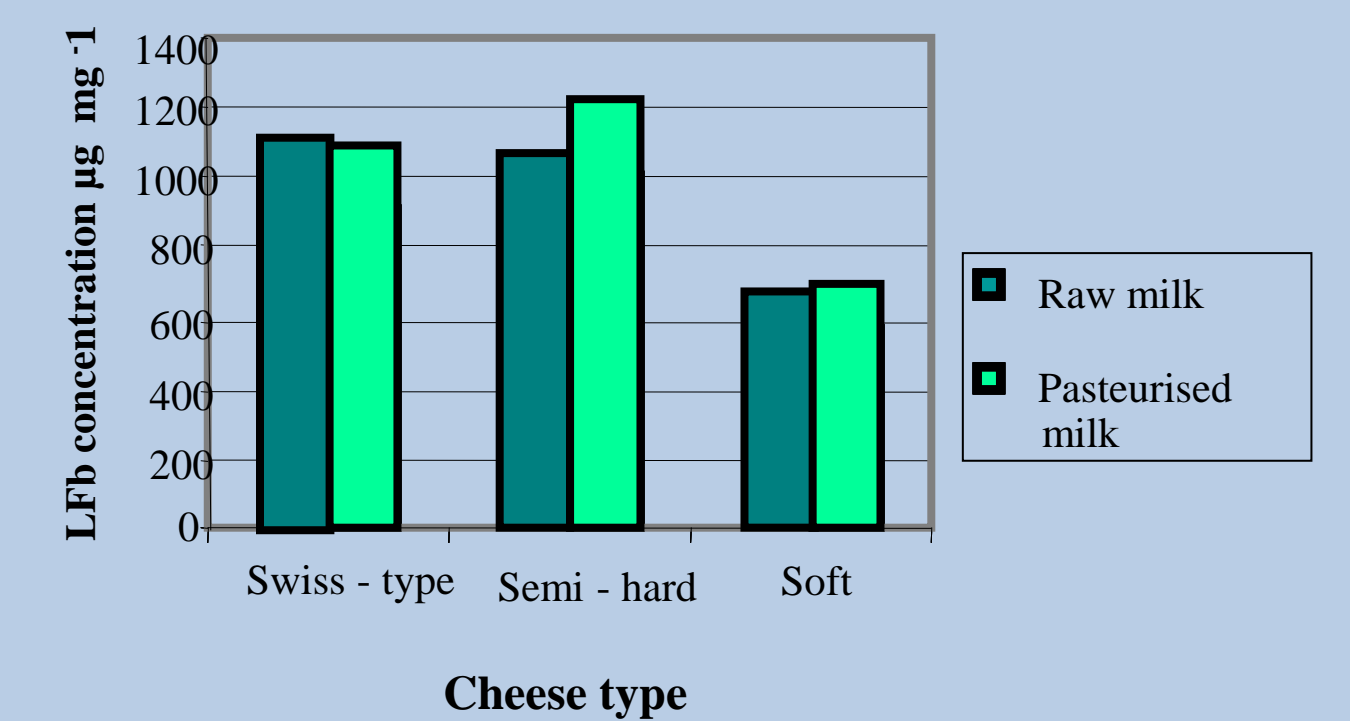
### Egg heating

Heating can affect structure of proteins, lead to partial hydrolysis or induce chemical modifications. Antibodies can be sensitive to these changes. Among the numerous antibodies directed against egg's proteins, some of them were selected for their abilities to detect both native and heated proteins (green circles).



### Cheese ripening

After selection of the appropriate antibodies a sandwich ELISA was developed for lactoferrin quantification in milk and cheese. Lactoferrin is present in all cheese types at the end of ripening in appreciable amounts.

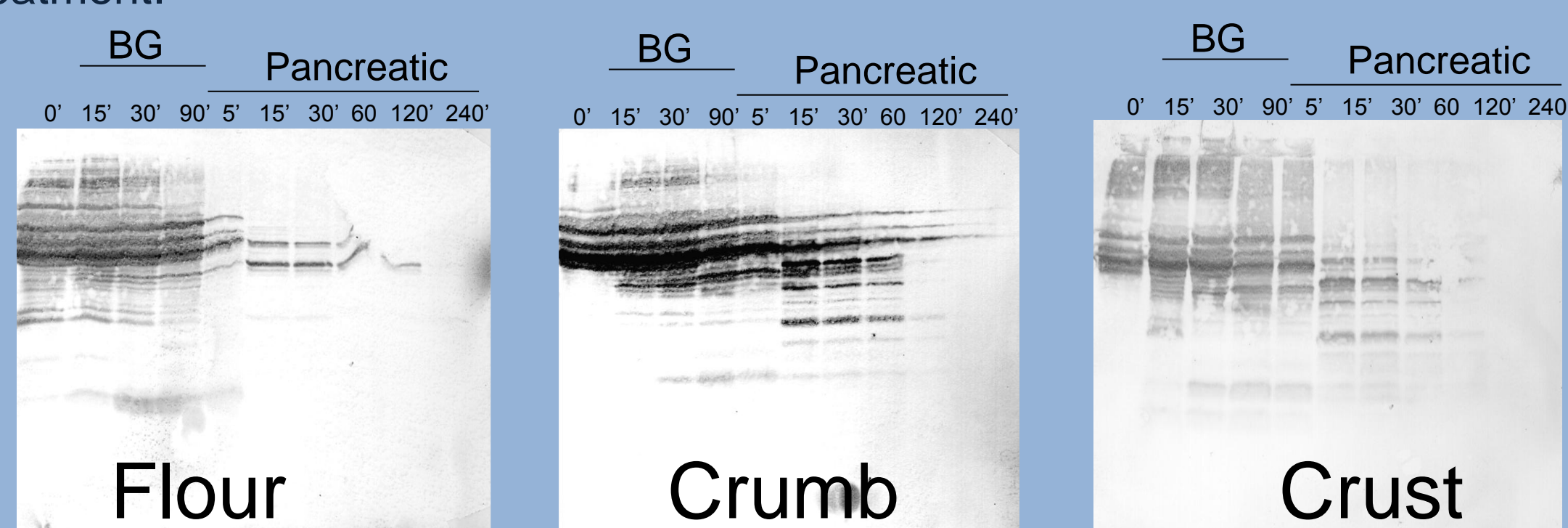


Dupont et al. 2006, Int Dairy J 16, 1081-1087

Antibodies enable the monitoring of the degradation of particular structure during digestion. Antibodies to wheat and milk were successfully used to evaluate the impact of some food process on the *in vitro* digestion of some peptides implied in food allergies to wheat and to milk.

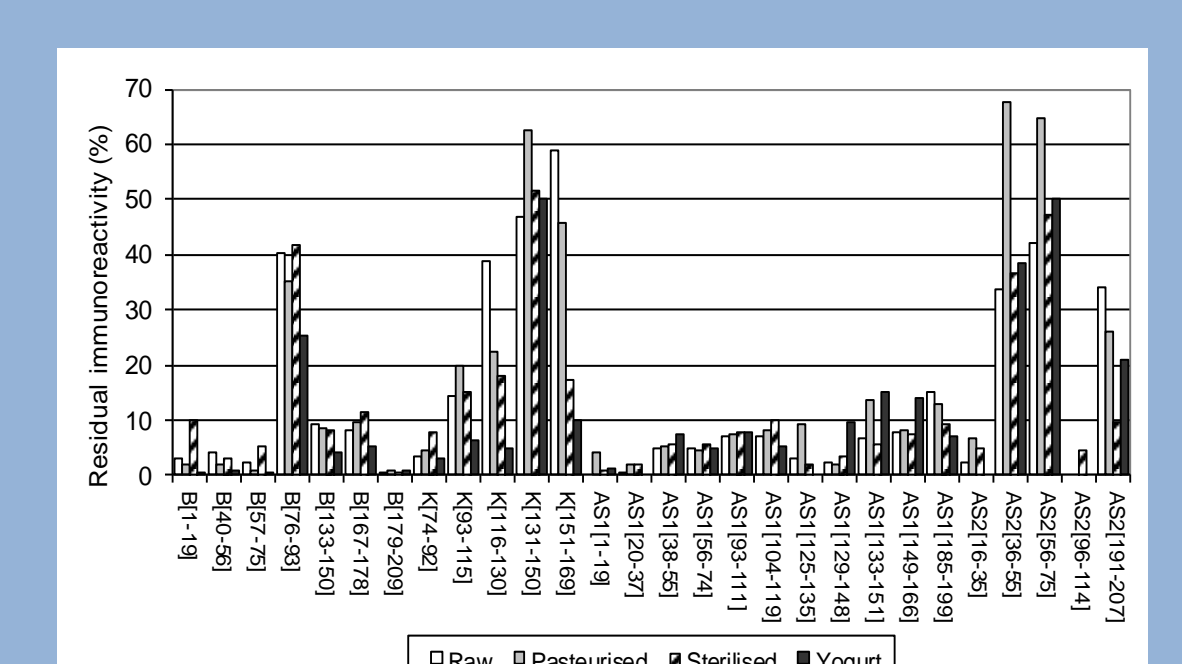
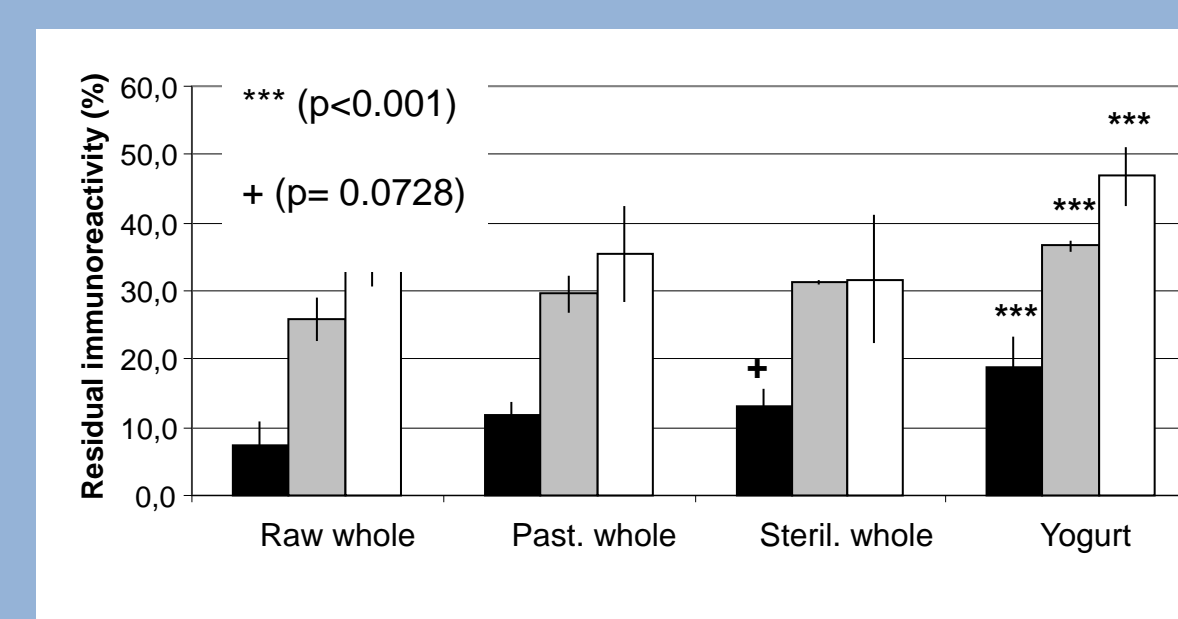
### Wheat digestion

Influence of heating on *in vitro* Bucco-Gastric (BG) and pancreatic digestion of wheat flour, bread crumb and crust was evaluated with antibodies specific to C-terminal peptide of β-gliadin by western blotting. Heating slow down the digestion but did not seem to change the immunoreactivity. Differences between crumb and crust were observed regarding the recognition of aggregated proteins. This point suggested that stability of proteins' aggregates is dependant on the heat treatment.



### Milk digestion

Different dairy matrices were manufactured and subjected to *in vitro* digestion using an infant gut model. Among other technics, hydrolysis of caseins (CN) was evaluated with immunochemical techniques. Residual immunoreactivity assessed by inhibition ELISA revealed that heat treatment increases the resistance of CN to infant digestion. Thanks to the set of monoclonal antibodies covering the sequence of caseins, parts of the different caseins which were resistant to digestion were identified.



Precise characterisation of the antibodies and their gathering in a unique collection make this antibodies' library a genuine toolbox to address raw material diversity, molecular complexity and the impact of process and digestion on food component and architecture.