INTEREST OF ANTI-PEPTIDE ANTIBODIES DIRECTED AGAINST GLIADINS TO DETECT SPECIFIC SEQUENCES AND EVALUATE CONFORMATIONAL CHANGES

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INTRODUCTION

We have obtained anti-peptide antibodies directed against the different classes of wheat prolamins some of which specifically detecting ω-gliadins (Denery-Papini et al., 1994). Omega-gliadins are thermostable proteins of great interest as they can be used as tracers in food products submitted to heating. The objectives of this study were to explore the interest of some of these anti-peptide antibodies to detect sequences specific of bread wheat and to follow the influence of thermal treatment on ω-gliadins.

EXPERIMENTAL : The reactivity of these antibodies against proteins contained in 50% propanol extracts of bread wheat flours and durum wheat semolina were analysed by immunoblotting after SDS-PAGE and by competitive ELISA. The influence of thermal treatments was examined by competitive ELISA. Pastas containing 10% of bread wheat were used as models. They were dried at different temperatures (60, 85 or 100°C) and additionnaly some of them were cooked in boiling water. Omega-gliadin and total gliadin contents of propanol extracts used in ELISA were determined by RP-HPLC.

Peptide	Antiserum	Reactivity
N-terminal : ARELNPSNKELGC	Anti-NT1- ω	Specific recognition of ω2 type-gliadins
Repetitive : PQQPYPQQPC	Anti-R-gliadin	Detection of some ω, γ and αβ-gliadins

Table I : Reactivity of the anti-peptide antisera used in the study.

SPECIFIC DETECTION OF BREAD WHEAT

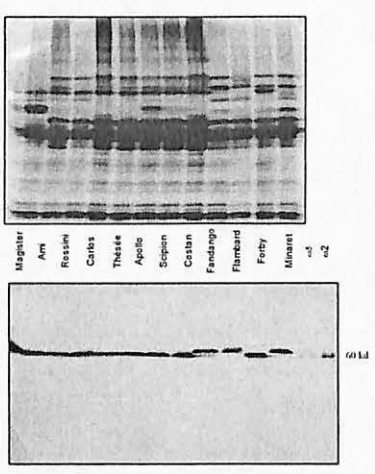


Figure 1 : Propanol extracts of **bread wheat flours** separated by SDS-PAGE a) Coomassie blue staining - b) immunoblotting analysis with anti-NT1-ω antiserum.

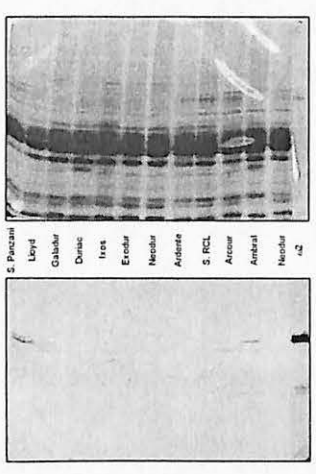


Figure 2 : Propanol extracts of **durum wheat semolina** separated by SDS-PAGE a) Coomassie blue staining - b) immunoblotting analysis with anti-NT1-ω antiserum.

The N-terminal sequences of ω2- type gliadins from bread wheat and from durum wheat differ by a single substitution at the third position (AREL → ARQL). Specific detection of bread wheat was possible by the anti-NT1-ω antiserum directed against the N-terminal peptide of bread wheat. The antiserum detected by immunoblotting a 60 kd component in the extracts of bread wheat flours (Fig 1) whereas it did not react with any proteins extracted from durum wheat semolina (Fig 2). Specific recognition of bread wheat by this antiserum was also observed in ELISA (Fig 3).

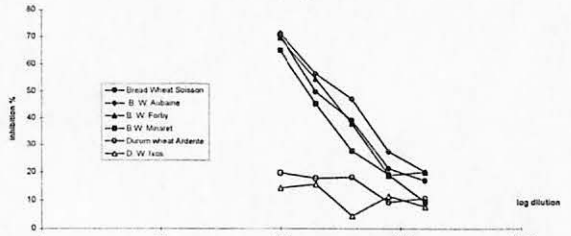


Figure 3 : Reactivity of the anti-NT1-ω antibodies in competitive ELISA with proteins extracted from various bread wheats and durum wheats.

INFLUENCE OF THERMAL TREATMENTS

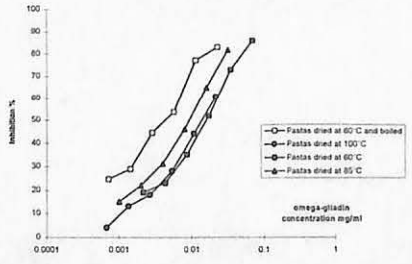


Figure 4 : Reactivity of the anti-NT1-ω antibodies in competitive ELISA with ω-gliadins from propanol extracts of different pasta samples.

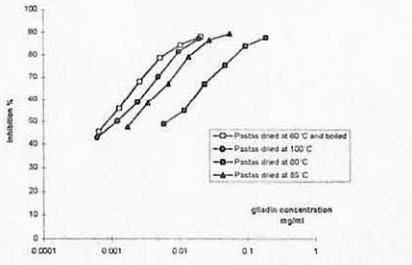


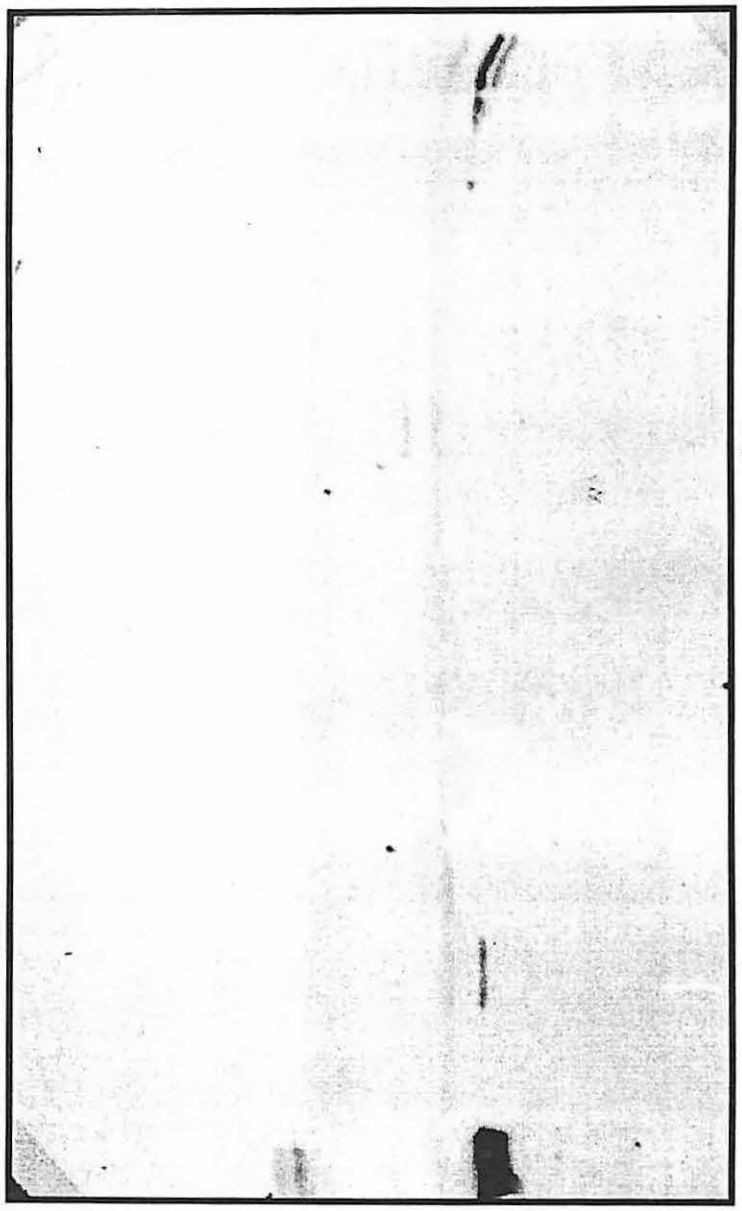
Figure 5 : Reactivity of the anti-R-gliadin antibodies in competitive ELISA with proteins from propanol extracts of different pasta samples.

No significant difference was observed with the anti-NT1-ω antibodies in the recognition of ω-gliadins from pastas dried at 60, 85 or 100°C ; it detected however better ω-gliadins from pastas submitted to a step of heating in boiling water (Fig 4). The anti-R-gliadin antibodies directed against a repetitive sequence of gliadins better recognized proteins extracted from pastas dried at high temperature (85 or 100°C) or from boiled pastas than those extracted from pastas dried at 60°C without boiling (Fig5).

CONCLUSION

Anti-peptide antibodies are generally directed against linear epitopes that are not so much affected by heat induced changes than conformational epitopes and therefore present an interest to detect and quantify proteins in food submitted to processing. We show in this study that the anti-NT1-ω and anti-R-gliadin antibodies can be used to observe structural modifications that occur in particular domains and that drying or heating differently affects N-terminal or repetitive regions. In addition, thanks to its narrow specificity, the anti-NT1-ω antiserum could be used to detect bread wheat additions in durum wheat semoulina and in pastas.

Fig 2



S. Panzani

Lioyd

Galadur

Duriac

Ixos

Exodur

Neodur

Ardente

S. RCL

Arcour

Ambral

Neodur

w2

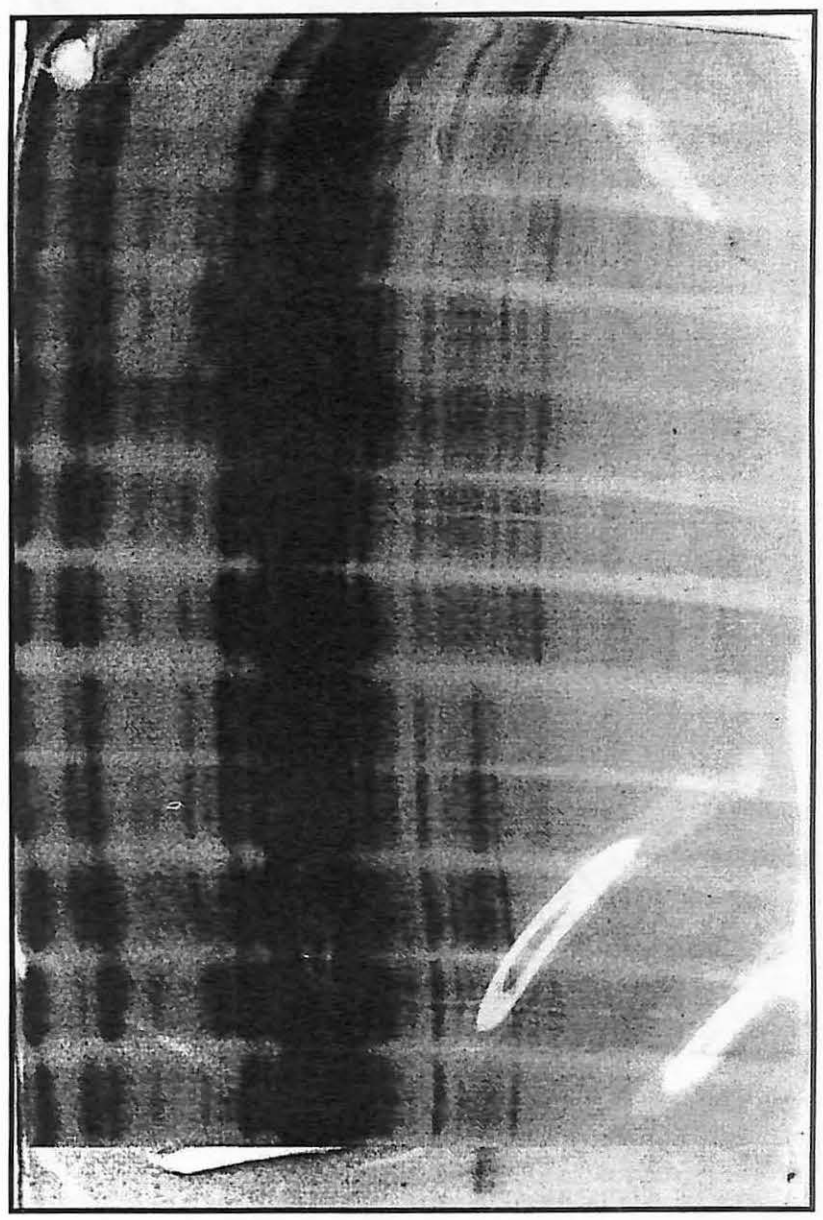


Fig 1

60 kd

Magister

Ami

Rossini

Carlos

Thésée

Apollo

Scipion

Costan

Fandango

Flambard

Forby

Minaret

ω5

ω2