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Anti-Peptide Antibodies Directed Against Omega-Gliadins for the Detection of Sequences from Bread and Durum Wheats*

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This paper presents the characterisation of polyclonal antibodies directed against two different N-terminal sequences of ω-gliadins. Both antisera recognised specifically the corresponding ω-gliadin fractions but showed different reactivities against ω-gliadins extracted from bread and durum wheats. Antibodies directed against the 'SRLL' type (ω5 type) recognised several ω-gliadin components in bread and durum wheat extracts whereas antibodies directed against the 'AREL' type (ω2 type) reacted specifically with an ω-gliadin component from bread wheat. The narrow specificity of this antiserum made it potentially interesting for the detection of bread wheat additions in durum wheat pasta. Moreover, the reactivity of this antiserum was not modified by an increase in drying temperature of pasta.

Keywords: *Anti-peptide antibodies, ω-gliadins, bread wheat, durum wheat*

INTRODUCTION

The ω-gliadin subgroup accounts for only a minor proportion (~10%) of total gliadins. However, unlike other prolamins, ω-gliadins possess the unique feature of not being involved in disulphide intra- or interchain reactions as they are cysteine-free. Therefore they are considered as heat-stable proteins whose extractability is less reduced by the cooking of food than that of other prolamin groups (Wieser, 1998). The stability of ω-gliadins to heating is of great interest as they can be used as prolamin tracers in various raw or cooked food products. On this basis Skerritt and Hill (1990) used a monoclonal antibody (MAb) directed against ω-gliadins to develop an immunochemical assay for gliadin determination in food and found

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TABLE 1. N-terminal sequences of ω-gliadins from bread and durum wheats (Kasarda *et al.*, 1983)

ω-gliadin types	Wheat species	Sequences
ω-1	*T. aestivum*	KELQSPQQ
	T. durum	KELQSPQQ
ω-2 ancestral type	*T. aestivum*	ARELNPSNKELQSPQQ
	T. durum	ARQLNP?N?E
ω-5	*T. aestivum*	SRLLSPRGKELHTPQQ

little variation in gliadin concentration before and after cooking of products. However, this MAb also gave cross-reactions with high molecular weight glutenins (Skerritt & Hill, 1990).

In the present study our objective was to obtain antibodies that could be used to specifically detect and quantify ω-gliadins in flour and evaluate the influence of this gliadin subgroup on the technological quality of wheat flour. In a previous work, we demonstrated that immunisation with synthetic peptides was an easy and efficient way to obtain antibodies that reacted only with a single prolamin group (Denery-Papini *et al.*, 1994); as they are specific for each prolamin group and easily accessible for antibody binding, N-terminal sequences were found to be particularly relevant immunogens. The same strategy was used in the present work. ω-gliadins display a polymorphism in their amino-acid composition, size and N-terminal sequences. On the basis of N-terminal sequences, Kasarda *et al.* (1983) classified these proteins as ω1, ω2 and ω5-gliadins (Table 1). Another classification based on mobility in acid-PAGE was also proposed by Popineau *et al.* (1986): slow and fast components were respectively named ω17, ω19 and ω28–30. On the other hand, an allelic classification was proposed by Khelifi *et al.* (1992), based on ω-gliadin mobility in SDS-PAGE: allele d11–d12 being the most abundant (cf. Capelle-Desprez or Hardi cultivars), in contrast to the allele d7. We already reported the production of polyclonal antibodies (PAbs) against the N-terminal peptide of ω2-type gliadins from bread wheat (Denery-Papini *et al.*, 1994); as similar sequences are also found in ω-gliadins from durum wheat, C-hordeins and ω-secalins the ω2 type is considered as an ancestral type. In order to widen the detection to other ω-gliadin types we immunised rabbits with the N-terminal peptide of ω5-gliadins. In this paper we describe the reactivity of these antisera with various prolamin fractions from bread and durum wheats and we give an example for potential application to the determination of semolina or pasta composition.

MATERIALS AND METHODS

Antigen Preparation

α, β, γ and ω-gliadin fractions and high and low molecular weight glutenin subunits were purified from Hardi cultivar as previously described (Denery-Papini *et al.*, 1994). The ω-gliadin fractions (ω17, ω19, ω28 and ω30) from Capelle-Desprez cultivar and A-PAGE slow and fast moving ω-gliadins from Hardi cultivar were obtained according to Popineau *et al.* (1986).

Pasta made with durum wheat semolina and containing 10% (w/w) of bread wheat were dried at different temperatures (60, 85 or 100°C). These samples were used as models to investigate the potential of antibodies for the detection of adulteration of durum wheat pasta by bread wheat.

Production of Antibodies
The NT2-ω peptide (SRLLSPRGKELGC) was synthesised and conjugated to ovalbumin (OVA) using m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as described by Denery-Papini *et al.* (1994). Antibodies against the conjugated NT2-ω peptide were raised in two month-old rabbits by subcutaneous injections. Production and characterisation of antibodies directed against the NT1-ω peptide (ARELNPSNKELGC) had been previously described (Denery-Papini *et al.*, 1994).

SDS-PAGE and Semi-dry Immunoblotting Procedures
ω-gliadins were extracted from 50 mg of durum wheat semolina or bread wheat flours by stirring during 1 h with 400 μl of 0.22 M-Tris-HCl buffer, pH 6.8, containing 2% (w/v) SDS and 20% glycerol. After centrifugation (15 min, 15 000 rpm) proteins (2.5 μl/well) contained in the supernatant were separated by SDS-PAGE on 12% acrylamide gels for 1.5 h at 15 mA/gel and then transferred to an Immobilon membrane in a semi-dry electroblotting apparatus. The immunodetection procedure was carried out as previously described (Denery-Papini *et al.*, 1994) and we used for the revelation step the alkaline-phosphatase conjugate substrate kit (Bio Rad) in 0.1 M-Tris HCl buffer, pH 9.6. The working dilutions were, respectively, 1/1000 and 1/2500 for the anti-NT1-ω and anti-NT2-ω antisera.

ELISA Procedures
The reactivity of the anti-NT2-ω antibodies with gliadin and glutenin fractions was analysed by antigen coated plate ELISA as described (Denery-Papini *et al.*, 1994). The reactivity of the anti-NT1-ω antibodies against ω-gliadins extracted from pasta was analysed by sequential competitive ELISA as follows. ELISA plates (Falcon 3915) were coated with an A-PAGE slow-moving ω-gliadin fraction (100 μl/well) at a concentration of 1 μg ml^{-1} in 0.05 M-carbonate buffer, pH 9.6, for 2 h at 37°C. The plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20. Unoccupied sites of the plastic plate were blocked with 200 μl/well of PBS containing 2% (w/v) freeze-dried low-fat milk for 1 h at 37°C. Gliadins were extracted from pasta samples (100 mg) with 500 μl of 50% propan-1-ol for 30 min at 60°C with three mixes at times 0, 10 and 20 min (Singh *et al.*, 1991). After centrifugation (15 min at 15 000 rpm) the supernatants were diluted in PBS, mixed in glass tubes with the anti-NT1-ω antibodies (diluted 1/1000) and incubated for 1 h at 37°C. Of the contents of each tube, 100 μl were then incubated in the ω-gliadin coated wells for 2 h at 37°C. After washing, alkaline phosphatase labelled goat anti-rabbit IgG (Sigma) diluted 1/3000 in PBS was added and incubated for 1 h at 37°C. After further washings, bound antibodies were revealed by addition of substrate *p*-nitrophenylphosphate in 0.1 M-diethanolamine–HCl buffer, pH 9.7. After 30 min of reaction, absorbance was measured at 405 nm. Absorbance values were expressed as inhibition percentages: $\%X = [1 - (A_X/A_{max}) \times 100]$, where A_{max} was the absorbance value in the absence of inhibitor.

Determination of ω-Gliadin Content by RP-HPLC
Before ω-gliadin extraction and quantification, albumins and globulins from ground pasta (100 mg) were washed by continuous stirring for 1 h at 4°C with 3 ml of 0.05 M-sodium phosphate buffer (pH 7.8), containing 0.1 M-NaCl. After centrifugation (5000 rpm for 10 min) ω-gliadin was extracted from the pellets with 50% propan-1-ol for 30 min at 60°C. After centrifugation the supernatants were diluted four-fold in 10% acetonitrile. Reverse-phase high-performance liquid chromatography (RP-HPLC) was performed on a Nucleosil 300 C18 column (5 μm, 300 Å, 250 × 4.6 mm) at 50°C. The two eluants were A: water containing 0.1% trifluoroacetic acid (TFA); B: acetonitrile and 0.08% TFA. The gradient was 0–3 min: 15% B; 3–63 min: 15–60% B; 63–64 min: 60–80% B; 64–68 min: 80% B; 68–70 min: 80–15% B; 70–80 min: 15% B. The flow rate was 1 ml min^{-1} and detection was carried out

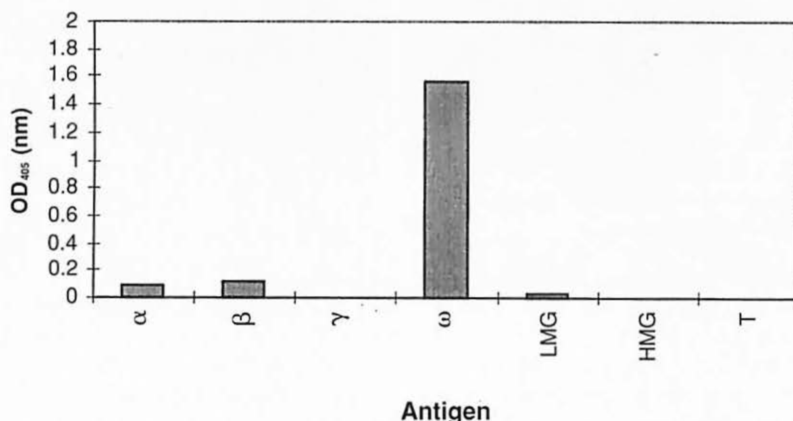

FIG. 1. Reactivity of anti-NT2-ω antiserum (diluted 1/5000) with various prolamin fractions (α, β, γ and ω-gliadins and low and high molecular weight glutenin subunits) analysed in antigen-coated plate ELISA. T: control without antigen on the plate.

at 220 nm. Injection volume was 110 μl. Freeze-dried purified ω-gliadins (1 mg/ml^{-1}) were used as a standard to determine ω-gliadin contents of propanol extracts. Omega-gliadins were quantified by integration of chromatogram areas between 20 and 36 min according to the ω-gliadin standard.

RESULTS

Reactivity of the Antisera with Various Prolamin Fractions from Bread Wheat

Anti-NT2-ω antiserum was characterised by ELISA with α, β, γ, ω-gliadin and low and high molecular weight glutenin subunits coated on the plates. The antiserum recognised only the ω-gliadin fractions and did not cross-react with any other prolamins (Figure 1). The same result was previously obtained with anti-NT1-ω antibodies (Denery-Papini *et al.*, 1994). Antisera were further analysed by ELISA with ω-gliadin components ω17, ω19, ω28 and ω30 purified from Capelle-Desprez cultivar and previously characterised (Popineau *et al.*, 1986). In electrophoresis at acid pH, components ω17 and ω19 corresponded to slow-moving ω-gliadins whereas ω28 and ω30 migrated faster. According to these electrophoretic mobilities and to amino-acid composition correspondence has been established between components ω17, ω19, ω28/30 and ω1, ω2 and ω5 types, respectively. As expected from the immunogen peptides, anti-NT1-ω antibodies detected only the ω19 component whereas anti-NT2-ω antibodies reacted specifically with ω28 and ω30 components (Table 2).

TABLE 2. Reactivity of antibodies directed against N-terminal sequences of ω-gliadins with ω-gliadin fractions purified from variety Cappelle-Desprez

		ω-gliadin fractions		
Antiserum	N-terminal peptide	ω17 ω1 type	ω19 ω2 type	ω28, ω30 ω5 type
anti-NT1-ω	ARELNPSNKELGC	–	+	– –
anti-NT2-ω	SRLLSPRGKELGC	–	–	+ +

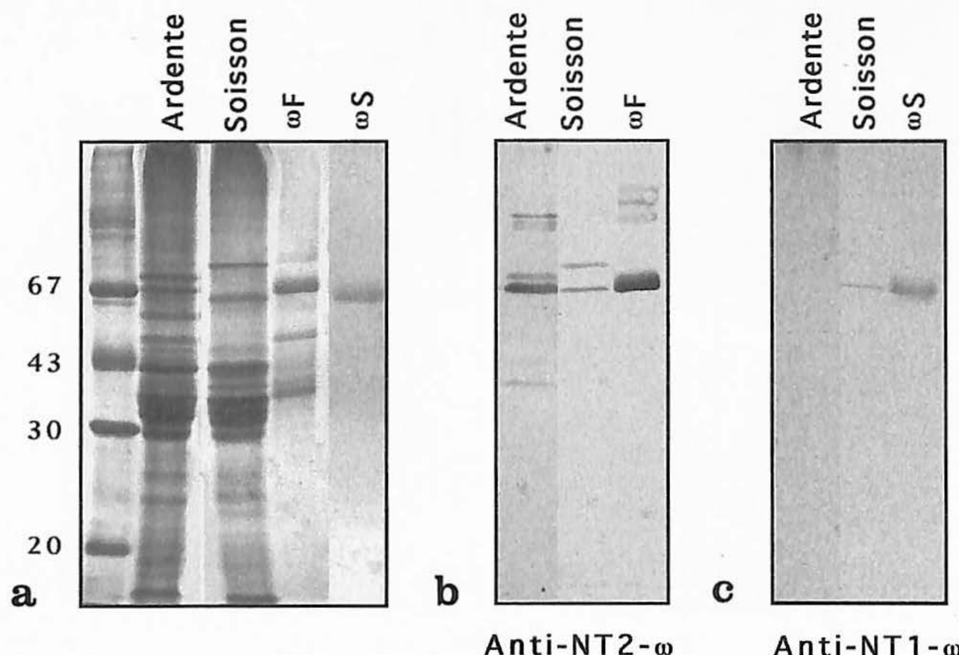

FIG. 2. Separation by SDS-PAGE of gliadins extracted from a bread wheat flour (Soissons) and a durum wheat semolina (Ardente) and of ω-gliadin fractions (ωF: A-PAGE fast-moving ω-gliadin; ωS: A-PAGE slow-moving ω-gliadin). (a) Coomassie blue stained gel; (b) and (c) immunoblotting analysis with anti-NT2-ω antiserum (diluted 1/2500) and anti-NT1-ω antiserum (diluted 1/1000), respectively.

Reactivity of the Antisera with ω-Gliadins from Bread and Durum Wheat
Antisera were tested by immunoblotting against gliadins extracted from a bread wheat flour (Soissons) and a durum wheat semolina (Ardente) and A-PAGE slow and fast-moving ω-gliadin fractions (ωS and ωF) from bread wheat Hardi (Figure 2). Anti-NT2-ω antiserum detected the fast-moving ω-gliadin control (ωF) as well as two components in extracts from bread and durum wheats. In the gliadin extract of Ardente cultivar, the antiserum detected a component with a molecular weight of 69 kDa and more strongly a component of 67 kDa both corresponding to major ω-gliadins after Coomassie blue staining. In the gliadin extract from Soissons cultivar, the antibodies reacted with a 72 kDa band which appeared as a major ω-gliadin and a 67 kDa band that could not be distinguished after protein staining. Anti-NT1-ω antiserum reacted with the A-PAGE slow-moving ω-gliadin control (ωS) and with a band of equal mobility in the bread wheat flour extract but detected no components in the durum wheat semolina extract. The ω-gliadin recognised in the extract of Soissons cultivar corresponded to a 61 kDa band well visible after Coomassie blue staining.

The specificity for bread wheat of the anti-NT1-ω antiserum was controlled by immunoblotting on a larger number of wheat varieties (examples given in Figure 3). No reaction was observed with proteins extracted from 24 durum wheat semolina whereas a component of 61 kDa was detected in 31 bread wheat flours and a band sligtly over 61 kDa in certain bread wheat varieties (Minaret, Flambard, Fandango) possessing the relatively rare d7 allele.

Influence of a Thermal Treatment on the Specific Detection of Bread Wheat
The narrow specificity of anti-NT1-ω antiserum for bread wheat ω-gliadins appeared interesting for the detection of bread wheat presence in durum wheat products as pasta. In

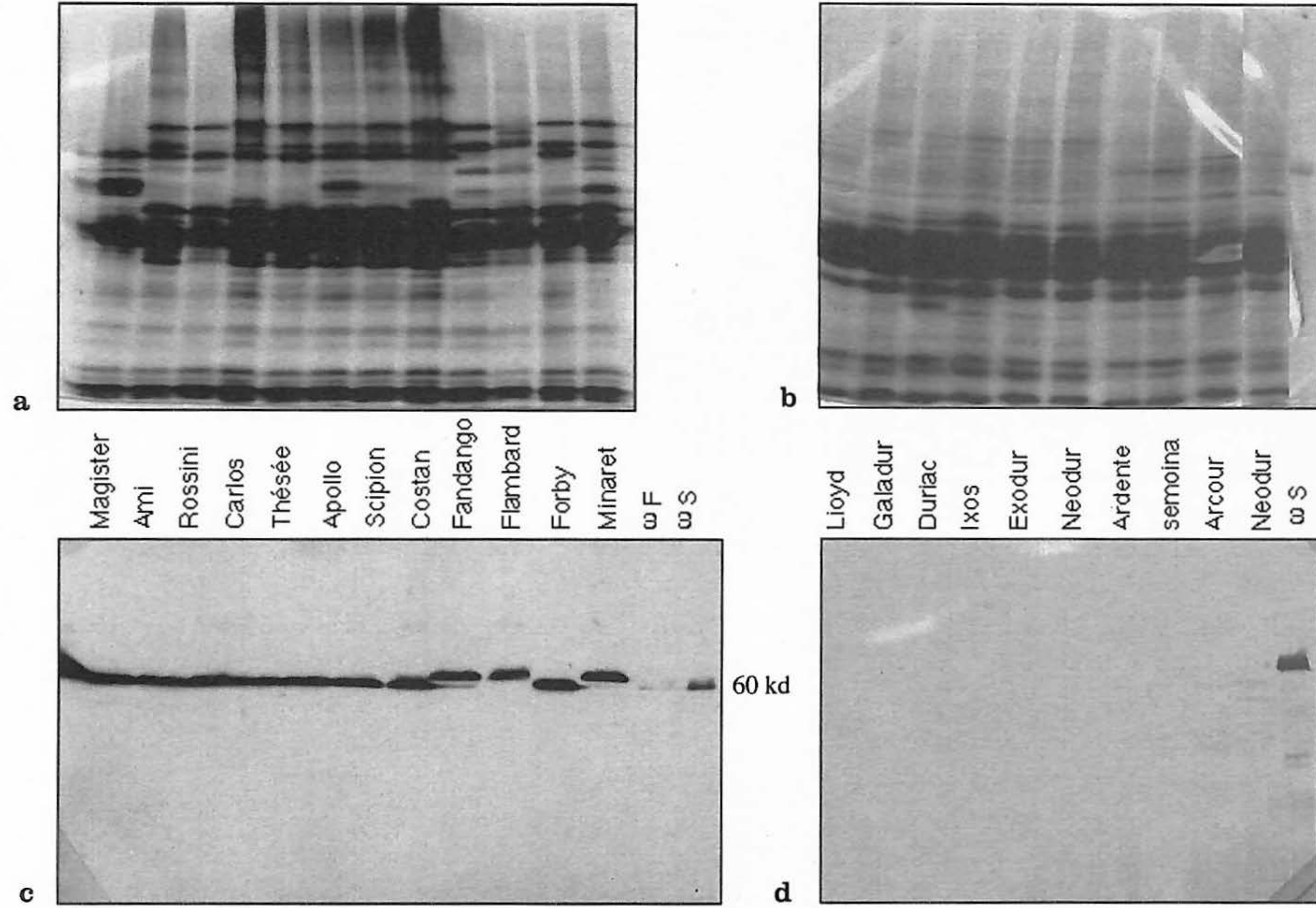

FIG. 3. Separation by SDS-PAGE of gliadins extracted from bread wheat flours (a) and (c) and durum wheat semolina (b) and (d). (a) and (b) Coomassie blue stained gels. (c) and (d) Immunoblotting analysis with anti-NT1-ω antibodies (diluted 1/1000).

TABLE 3. ω-Gliadin content determined by RP-HPLC of 50% propanol extracts from pasta dried at three different temperatures

Drying temperature (°C)	Concentration (mg ml^{-1})	Total proteins (%)
60	0.87	9
85	0.40	15
100	0.27	27.5

view of using this antiserum on dried pasta, we have studied the influence of the drying temperature on antibody response. Pasta containing 10% bread wheat and dried at three different temperatures (60, 85 and 100°C) were used as models. The ω-gliadin content of propanol extracts from these samples were determined by RP-HPLC. Table 3 shows that ω-gliadin extractability was lowered by an increase of drying temperature; however, their extractability was less reduced than that of other gliadin groups. The reactivity of anti-NT1-ω antibodies with proteins contained in propanol extracts was analysed by competitive ELISA. At equal ω-gliadin content in extracts no significant difference was observed in the detection of these proteins from pasta dried at 60, 85 and 100°C (Figure 4).

DISCUSSION

In the present work we confirmed that immunisation with N-terminal peptides of ω-gliadins induced antibodies that recognised the homologous proteins without cross-reaction with other gliadin or glutenin groups. In the case of ω-gliadins, the polymorphism of N-terminal sequences led us to produce PAbs against two different N-terminal peptides, AREL and

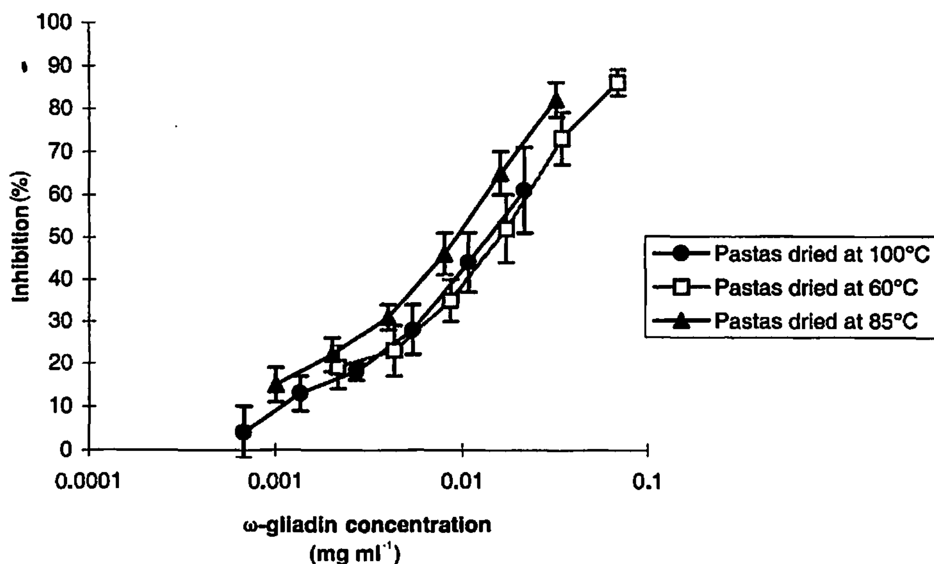

FIG. 4. Reactivity of anti-NT1-ω antibodies in competitive ELISA with proteins extracted from different pasta samples (data represent means from assays on four different days).

SRLL types, that reacted specifically with the corresponding ω-gliadin types ω2 and ω5. The SRLL type sequence was detected both in bread and durum wheats and corresponded to several ω-gliadin components. On the contrary, the AREL sequence was only detected in bread wheat varieties and corresponded to only one ω-gliadin band. N-terminal sequences of ω2 type gliadins from bread and durum wheats differ by a substitution at position 3 (AR*E*LNP → AR*Q*LNP) that corresponds to the replacement of an acidic group by a polar neutral group. As already seen in previous work with anti-peptide antibodies against γ-gliadins and HM_rG subunits (Denery-Papini *et al.*, 1994; 1996) this substitution made it possible to observe with anti-NT1-ω antiserum, in immunoblotting, a very narrow specificity for bread wheat ω2-gliadins.

Incorporation of bread wheat in pasta is generally due to economic reasons but leads to lower quality products (aspect, cooking) than 100% durum wheat pasta. The European Commission recently supported a programme (Autran *et al.*, 1998) aimed at developing methods for the control of pasta composition and labelling. Barnwell *et al.* (1994) used RP-HPLC to observe a mixture of γ and β-gliadins found specifically on bread wheat chromatographic profiles. A Durum Test Immunoassay was developed based on a MAb against friabilin (Mackay & Stimson, 1993); this protein is encoded on chromosome 5D of hexaploid wheats and is not found in durum wheats. Autran and Bonicel (1992) proposed to detect slow-moving ω-gliadins encoded on chromosome 1D of bread wheat by electrophoresis at acid pH. However, the sensitivity and accuracy of these methods were affected by drying temperatures, genetic variability or additives (as eggs) incorporation in pasta. As high drying temperatures (100°C) are generally used in the pasta industry, heat resistant ω-gliadins appeared as interesting bread wheat tracers in such products. Stevenson *et al.* (1994) produced PAbs against A-PAGE slow-moving ω-gliadins specific for bread wheat and purified by RP-HPLC. However, because of the extensive sequence homologies between gliadins from bread and durum wheat these anti-protein antibodies cross-reacted with both wheat species. On the contrary, thanks to their narrow specificity anti-peptide antibodies against the N-terminal peptide from bread wheat ω2-gliadins seemed interesting for the detection of bread wheat additions in durum wheat products. Hence, we tested the influence of an increase in drying temperature (from 60 to 100°C) on the antigenicity of the ω-gliadin N-terminal region. Antibody accessibility to this N-terminal epitope was not modified by temperature augmentation. Moreover, anti-peptide antibodies are generally directed to linear epitopes that are less affected by conformational changes than conformational epitopes; they are of interest to the detection of proteins in technological treatments. However, as shown in the work of Wieser (1998), we observed that ω-gliadin extractability in aqueous propanol was altered by an increase of drying temperature. This lowering in ω-gliadin concentration due to the extraction procedure will thus induce errors in quantification tests.

CONCLUSION

In this paper we have described the reactivity of anti-peptide antibodies directed against two ω-gliadin types (ω2 and ω5) that could be used to characterise, detect or quantify these proteins in flour or in food products. Antibodies against the N-terminal peptide 'ARELNPSNKEL' recognised specifically bread wheat ω2-gliadins and could be used to develop a method for the detection and quantification of bread wheat additions in durum wheat pasta.

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