Use of Specific Polyclonal Antibodies to Detect Heat Treatment of Ovalbumin in Mushrooms

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ABSTRACT

Using ovalbumin as a model protein in pure solution it was found that affinity chromatography of antisera against either native ovalbumin (NOA) or heat-denatured ovalbumin (HDOA) gave four different antibody populations AC1-4 from each antiserum, with different binding properties to the related or unrelated antigen. Direct ELISA was shown to be useless for differentiating NOA from HDOA. Immunoprecipitation in solution is time consuming and, moreover, whereas NAC1 was shown to be specific for NOA compared with HDOA, DAC1, NAC2 and DAC2 were shown not to be fully specific for HDOA. In contrast, by using as capture antibodies either NAC3 or DAC4, a sandwich ELISA can be designed which is fully specific for NOA or HDOA, respectively. An approach to the identification of the temperature at which ovalbumin has been heated is described. This test will show whether ovalbumin has been heated to lower than 65°C or higher than 85°C. The test was applied to juices from canned mushrooms containing 2% ovalbumin.

Key words: Food quality, native, heat-denatured, ovalbumin, mushrooms, immunoprecipitation, ELISA, HPLC.

ABBREVIATIONS

ABTS 2,2-Azino-di-(3-ethylbenzthiazoline-6-sulphonate)

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The detection of added proteins in food has been widely approached in the industry by immunochemical techniques (Griffiths et al. 1981, 1984; Skerrit and Smith 1985; Breton et al. 1988a). However, these techniques have so far been limited for a number of different reasons. (a) Proteins in foods are mostly denatured because of the physical, chemical and enzymic transformations undergone during food processing; thus, epitopes found on native molecules are often modified or destroyed. (b) Depending upon the type of immunoassay used, different conclusions can be drawn when a given antigen is present (Breton et al. 1988a, 1989); native ovalbumin is fully precipitated by polyclonal antibodies whereas the latter will not bind NOA-coated plates.

It is becoming important to study epitopes transformed during food processing to identify and quantify components, and also additives, and thus provide for improved quality control. The utilisation of proteins in food is largely determined by their functional properties such as emulsifying, stabilising, foaming and gel-forming properties. Egg albumin is one of the most important proteins in food products. As reported by Shimada and Matsushita (1980), heat coagulation of ovalbumin results from the formation of disulphide links and the exposure of hydrophobic groups. Ovalbumin is a good model for such studies because the presence of disulphide bridges limits the extent of denaturation. This might explain why common epitopes were found in this work to both the native (NOA) and the heat denatured (HDOA) molecules (Breton et al. 1989). Similarly, human anti-ovalbumin IgE antibodies were found to bind both the native and the denatured forms of ovalbumin after digestion (Elsayed et al. 1986). Kilshaw et al. (1986), by using monoclonal antibodies of the IgG1 subclass, concluded that important differences characterised epitopes of NOA compared with those of HDOA; however, only four monoclonals were used to reach this conclusion. Some thermal treatments, including extrusion cooking, are likely to modify completely epitopes found on the native molecule. It is thus necessary to investigate specific epitopes for the denatured form of the molecule. It was shown, for instance, that glycosylation of ovalbumin through the Maillard reaction takes place by heat treatment at low temperature, leading to a loss of immunogenic properties (Kato et al. 1985).

It is also important to evaluate the accuracy of different immunochemical methods to quantify NOA and HDOA, in order to control or to identify ovalbumin in heat-processed foods containing egg. In this presentation we shall describe
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different antibodies obtained from rabbits immunised against either NOA or HDOA and purified by affinity chromatography. It is shown that, in sandwich ELISA, such antibodies can distinguish NOA from HDOA and can be used for the recognition of the temperature at which canned mushrooms have been heated. This new approach may be helpful in characterising and quantifying proteinaceous components in food, with a possible assessment of the temperature range at which the food has been heated.

EXPERIMENTAL

Hen egg albumin (ovalbumin fraction VII) was purchased from Sigma Chemicals (St Louis, MO, USA). Ultrogel ACA-22 was obtained from IBF Reactifs (Villeneuve la Garenne). Immunoplates for ELISA were from Nunc (Intermed, Noisy le Grand). Peroxide-coupled goat anti-rabbit IgG was obtained from Bio-Rad Laboratories (Paris) and peroxidase substrate ABTS (2,2-azino-di-(3-ethylbenzthiazoline-6-sulphonate)) was from Boehringer Mannheim, Mannheim, FRG). Canned mushrooms with 20 g kg⁻¹ additive ovalbumin, treated at different temperatures (50, 65, 85 and 100°C) for 10 min, were provided by M. Duclos (ANICC, Saint-Paterno).

Unlabelled and labelled ovalbumin

Native and heat-denatured ovalbumin were prepared according to the method described earlier (Breton et al 1989). NOA and HDOA were labelled with ¹²⁵I using Bolton-Hunter reagent. Specific activities were 11·1 kBq µg⁻¹ NOA and 3·1 kBq µg⁻¹ HDOA.

Immunisation

Rabbits (3 months old) were immunised by multiple intradermal injections of antigen (either NOA or HDOA) in Freund’s complete adjuvant. Each rabbit received 1 mg antigen per injection. Two weeks later the procedure was repeated with incomplete adjuvant. Animals were bled 5 to 7 days after the last injection. Hyperimmunisation took place once a month by intravenous injection of 1 mg antigen in 0·5 ml saline followed by bleeds on days 5, 10 and 15. Mice were immunised with HDOA as described above except that antigen doses were adjusted at 30 µg per injection.

Preparation of affinity purified antibodies

(a) Affinity columns

NOA and HDOA was coupled to Ultrogel ACA-22 as previously reported (Breton et al 1989).

(b) Antibody purification

A 2-ml aliquot of either rabbit anti-NOA or anti-HDOA was loaded successively on to NOA and HDOA columns, or vice versa according to Fig 1 in order to obtain NAC₁–NAC₄ from anti-NOA serum and DAC₁–DAC₄ from anti-HDOA serum.
Antibodies were eluted with 0.2 M glycine–HCl buffer pH 2.3 containing 10% dioxane, immediately neutralised with crystallised Tris and then dialysed against cold phosphate buffer saline (PBS).

Enzyme linked immunosorbent assays (ELISA)
These were performed as previously described (Breton et al 1989).

Direct ELISA
Microtitre plates (Nunc) were incubated with 0.2 ml (200 ng ml⁻¹) of antigen (NOA, HDOA or mushrooms treated at different temperatures), and a 50 g litre⁻¹ solution of dry skim milk was used for plate saturation. Plates were incubated with affinity purified antibodies (NAC₁–NAC₄ or DAC₁–DAC₄) for 2 h at 37°C or overnight at 4°C.

Sandwich ELISA
In brief, the first incubation was made with 0.2 ml (200 ng ml⁻¹) of affinity purified antibodies (NAC₁–NAC₄ or DAC₁–DAC₄). After antigen fixation, plates were incubated with the second antibody made by 1 + 999 dilution of mouse antiserum against either NOA or HDOA. The third antibody was a goat peroxidase-labelled anti-mouse serum. The presence of enzyme was revealed with ABTS.
Immunocapture of $^{125}$I-NOA and $^{125}$I-HDOA by affinity purified polyclonal antibodies

Microtitre plates were coated with 0.2 ml of a solution of 1 μg of affinity purified polyclonal antibodies (NAC₁–NAC₄ or DAC₁–DAC₄) in 1 ml pH 9-6, 0.05 M carbonate buffer at 37°C for 2 h. Plates were washed and blocked as described above. After washing, plates were incubated with 0.2 ml of radiolabelled antigen $^{125}$I-NOA or $^{125}$I-HDOA (1 μg ml⁻¹ in PBS) overnight at 4°C. Wells were washed four times with PBS–Tween 20, cut and counted, and the amount of antigen captured was calculated.

Quantitative immunoprecipitation

The standard procedure as described previously (Breton et al 1989) was employed. In brief, fixed amounts of purified NAC₂, DAC₁ or DAC₂ were mixed with increasing concentrations of either NOA or HDOA (10–200 μg) to a final volume of 1 ml PBS. Succeeding steps were as described previously.

High performance liquid chromatography (HPLC)

A gel filtration HPLC column TSK 4000 SW (600 x 7.5 mm) was used with a Spectra Physics model 8700 liquid chromatograph equipped with a UV/visible SP 8440 variable wavelight detector connected to a 4200 computing integrator (Gilson, Villiers Le Bel).

Samples (200 μl) were injected on to the column. The elution was effected with phosphate buffer (pH 7) containing 0.175 M NaCl at a rate of 1 ml min⁻¹. The eluates were monitored at 220 nm.

Identification of heat-treated ovalbumin in canned mushrooms

Ovalbumin (20 g kg⁻¹) was added to experimental canned mushrooms and the procedure was as described previously (Breton et al 1988b). Heat treatment of these cans was carried out at 50, 65, 85 and 100°C for 10 min. Ovalbumin coagulated at either 85 or 100°C was solubilised as in Breton et al (1989). Affinity purified polyclonal antibodies of different specificities (200 μl of 0.2 mg ml⁻¹ solutions) were first coated on plates. After incubating either with pure ovalbumin solutions or mushroom extracts, the presence of ovalbumin was detected by antibodies as described in the section headed ‘Direct ELISA’.

Protein estimation

Protein concentrations were determined either spectrophotometrically using extinction coefficient $E_{280\text{nm}}^{1\%} = 0.72$ for ovalbumin and 1.4 for antibody or colorimetrically by the Bradford method (Bradford 1976).

RESULTS

(1) Antibody analysis by affinity chromatography

The preparation of eight different antibodies by affinity chromatography is shown in Fig 1 and Table 1. The controls were when (1) sera from non-immunised rabbits
TABLE 1
Antibody specificities in rabbit antisera as judged by affinity chromatography (results are from a typical experiment; weights per 2 ml of serum)

|       | $AC_1$  | $AC_2$  | $AC_3$  | $AC_4$  |
|-------|---------|---------|---------|---------|
| Anti-NOA | 1.56 mg | 0.28 mg | 1.45 mg | 0.13 mg |
| Anti-DOA | 1.07 mg | 0.95 mg | 0.37 mg | 0.28 mg |

were applied to either NOA or HDOA columns, no immunoglobulin bound to these columns; (2) no detectable antibodies were found in the flow-through fluids after passing immune sera through both columns. In anti-NOA serum, $NAC_3$ were considered as specific for NOA and $NAC_4$ specific for HDOA, and the total antibody concentration was 1.70 mg ml$^{-1}$ ($NAC_1 + NAC_4/NAC_2 + NAC_3$). Thus, as judged by affinity chromatography, anti-NOA sera contained antibodies mainly directed against NOA, namely $NAC_3$ (85%) and antibodies directed against DOA, namely $NAC_4$ (7%). Antibodies against both forms represented only a small fraction of the total. In anti-HDOA serum, $DAC_3$ were specific for NOA and $DAC_4$ were specific for HDOA, and the total antibody concentration was 1.35 mg ml$^{-1}$ ($DAC_1 + DAC_4/DAC_2 + DAC_3$). Thus in the anti-HDOA antisera, 50% of antibodies ($DAC_1 - DAC_2$) were directed against common epitopes to NOA and HDOA while only 22% were specific for HDOA ($DAC_4$) and 28% for NOA ($DAC_3$).

(2) Antibody analysis by immunoprecipitation in solution followed by ELISA and HPLC

The addition of any amount (up to 200 µg) of $DAC_1$ antibody to NOA gave rise to a small amount of immunoprecipitate, while an increased precipitation was observed with HDOA (Fig 2a). After removal of immunoprecipitates by high speed centrifugation, supernates were shown by direct ELISA to contain mainly antibodies directed against HDOA. This is shown by the slope obtained against NOA as compared with that of HDOA (Fig 2b) on HDOA-sensitised plates. (NOA-sensitised plates were not suitable for analysis, since fixed NOA underwent drastic conformational change as reported by Breton et al (1989).) Whereas the addition of 40 µg of HDOA was enough to inhibit 85% of the ELISA response, the addition of 200 µg of NOA inhibited only 35%. When those supernates were studied by HPLC (Fig 3), at low dose (20 µg ml$^{-1}$) of either NOA (Fig 3a) or HDOA (Fig 3c) as antigen, a large peak of free antibody was seen (20.1) in both cases. This fitted the above observation that the immunoprecipitation did not take place with NOA whereas it was incomplete with HDOA at 20 µg ml$^{-1}$. At high dose of antigen (200 µg ml$^{-1}$) a large increase of NOA was seen (20.8) (Fig 3b) whereas with HDOA only a small amount of free antibody (20.1) and free antigen (9.5) can be seen (Fig 3d). This was in accordance with the observation that a very small amount of precipitate was recovered in the presence of NOA whereas a large amount of precipitate was recovered with HDOA. Thus $DAC_1$ binds both NOA and HDOA but binds HDOA more strongly than NOA in solution. In Fig 3b, peaks 10.2 and 17.2 are due to IgM–NOA and IgG–NOA complexes respectively (see below).
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A similar study of NAC2 and DAC2 by immunoprecipitation (Figs 4a and 4b), ELISA (Figs 4c and 4d) and HPLC (Figs 5a–5d) showed that both antibodies were essentially directed against HDOA. For NAC2, HPLC analysis (Fig 5a) showed that, with a small amount of added NOA, free antibodies (20.1) with immune complexes at 10.2 (IgM) and at 17.5 (IgG) were present. At a high dose of NOA the two peaks of immune complexes increased along with free NOA (20.8). In contrast, with HDOA at low dose only, a small peak of free antibodies was detected whereas at a high dose a peak of HDOA was detected (9.5). For DAC2 the profile was quite different: in the presence of small amounts of NOA only free antibodies were found (20.1) whereas an excess of NOA gave a large peak of free NOA (20.8) with an important shoulder of free antibodies (20.1). Supernate of DAC2 with a low dose of HDOA showed a small amount of free antibody, and a small peak of free antigen was found with a large dose of HDOA.

Thus NAC2 bound but did not precipitate NOA (as evidenced by immune soluble complexes) while precipitating HDOA. Similar studies cannot be done with NAC3 as ELISA could not be used to measure free antibodies (see Table 2) or with NAC4 (due to poor recovery).
Fig 4. Immunoprecipitation of NAC₂ and DAC₂ antibodies. The experiment was performed as in Fig 2. (a) and (b) weights of precipitated protein from NAC₂ and DAC₂ respectively; □ with HDOA; ◆ with NOA. (c) and (d) Supernate studied by direct ELISA from NAC₂ and DAC₂ respectively, □ with HDOA; ◆ with NOA.

(3) **Comparison of affinity purified polyclonal antibodies by ELISA**

It can be seen (Table 2) that NAC₃ did not bind either NOA or HDOA on plates. Only NAC₄ seemed to be perfectly specific for HDOA. All DAC antibodies were non-specific, recognising both NOA and HDOA.

(4) **Comparison of affinity purified polyclonal antibodies by immunocapture**

Table 3 shows that NOA was captured best by NAC₁ and NAC₃ (3.7 and 2.6 ng respectively) as expected, but these antibodies (and especially NAC₁) also captured HDOA. The largest amounts of HDOA captured were by NAC₂, NAC₁, DAC₃, DAC₄, NAC₃ and DAC₂, in decreasing order of importance. Most important is the fact that NAC₃ is the most specific for NOA whereas DAC₄ is completely specific for HDOA. These results were confirmed by sandwich ELISA (Table 3), but depending on the mouse antibody used because second antibody signals can be non-specific. For instance, with NAC₃ used for antigen capture, the mouse anti-NOA serum gave an OD of 1.5 for NOA and of 0.7 for HDOA, while the mouse anti-HDOA serum gave an OD of 0.4 and 0.0 respectively. Similarly, with DAC₄ used for capture, mouse anti-NOA gave 0.5 for both antigens whereas the mouse anti-HDOA distinguished NOA (0.0) and HDOA (0.6). Thus in both cases the
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Fig 5. HPLC analysis of NAC2 (a, b, c, d) and DAC2 (e, f, g, h) supernate after interaction with either NOA at low concentration (a, c) or high concentration (b, f) or with HDOA at low concentration (c, g) or high concentration (d, h). Elution times as in Fig 3.

Mouse anti-HDOA was shown to be more suitable for discrimination between captured NOA and HDOA.

(5) Recognition of ovalbumin in canned mushroom as a function of heat treatment depending upon polyclonal antibodies used

Either NAC3 (mostly specific for NOA) or DAC4 (specific for HDOA) was coated on a plate at 200 ng ml⁻¹. Juices from canned mushroom (with 2% ovalbumin used
TABLE 2
Comparison of affinity purified polyclonal antibodies as studied by direct ELISA. Results are expressed as optical densities after 30 min incubation with substrate

|        | NAC₁ | NAC₂ | NAC₃ | NAC₄ | DAC₁ | DAC₂ | DAC₃ | DAC₄ |
|--------|------|------|------|------|------|------|------|------|
| NOA    | 1.5  | 1.1  | 0    | 0    | 1.5  | 1.5  | 0.6  | 2    |
| HDOA   | 1.5  | 1.5  | 0    | 0.5  | 1.7  | 2    | 1.2  | 2    |

TABLE 3
Capture of NOA and HDOA by polyclonal antibodies

| Weight captured³ | Absorbance² |
|------------------|-------------|
| ¹²⁵I-NOA         | ¹²⁵I-HDOA   |
| NOA              | HDOA        |
| Mouse anti-NOA  | Mouse anti-HDOA |
| NAC₁            | 3.7         | 22          |
| NAC₂            | 1.6         | 37          |
| NAC₃            | 2.6         | 5.3         |
| NAC₄            | 0.0         | 2.0         |
| DAC₁            | 1.0         | 3.4         |
| DAC₂            | 1.0         | 5.3         |
| DAC₃            | 0.8         | 11          |
| DAC₄            | 0.0         | 10          |

|         |         | Mouse anti-NOA | Mouse anti-HDOA |
|---------|---------|----------------|-----------------|
| NAC₁    | 1.7     | 0.7            | 0.8             |
| NAC₂    | 1.6     | 0.7            | 0.7             |
| NAC₃    | 1.5     | 0.4            | 0.4             |
| NAC₄    | 0.4     | 0.0            | 0.4             |
| DAC₁    | 0.4     | 0.0            | 0.4             |
| DAC₂    | 0.4     | 0.0            | 0.4             |
| DAC₃    | 0.5     | 0.0            | 0.5             |
| DAC₄    | 0.5     | 0.0            | 0.5             |

³Expressed as ng labelled antigen captured by well after coating with a standard weight of antibody.
²Expressed as OD after 30 min incubation with the substrate.

as additive) which had been heated at different temperatures were used as antigens on each plate. Mouse anti-HDOA antibodies were used to reveal the presence of captured ovalbumin. All controls (canned mushrooms without added ovalbumin) were negative; it can be seen in Table 4 that drastic changes of NOA conformation occurred between 65 and 85°C. In that temperature range, by using NAC₃ for immunocapture it was possible to detect the presence of native OA heated up to 65°C, whereas with DAC₄ antibodies it was possible to recognise the ovalbumin that had been heated at 85°C or 100°C.

Thus heat treatment of food could be detected by this method.

DISCUSSION

Immunochemical analysis of food products is becoming more and more useful for a variety of purposes. In most instances only one technique has been performed. In this presentation it has been shown that antibodies with different specificities
Identification of heat-treated ovalbumin in canned mushrooms based on optical densities from ELISA procedure using mouse anti-HDOA antiserum (1:1000) as revealing antibody

| Capture antibody | Temperature of heat treatment (duration, 10 min) |
|------------------|--------------------------------------------------|
|                  | Untreated  | 50°C      | 65°C      | 85°C      | 100°C     |
| NAC₃             | 0.500      | 0.500     | 0.500     | 0.0       | 0.0       |
| DAC₄             | 0.0        | 0.0       | 0.0       | 0.700     | 0.700     |

present in an anti-serum can be separated by affinity chromatography. However, the final specificity will be dependent upon the technique used.

Several reports have shown that ovalbumin in heated food can be detected (Janssen et al. 1987) and quantified (Breton et al. 1988b) by immunochemical methods, but until now no experiment has been reported where an immunochemical test could be used to determine whether the product has been heated in the range ‘native or heated to below 65°C’ or ‘heated at 85 to 100°C’. We have designed a method by which several polyclonal antibodies could be used by immunising rabbits and mice with either native or heat-denatured ovalbumin and by selecting specific polyclonal antibodies by affinity chromatography on either NOA or HDOA columns. NOA is a monomer of MW 43 kDa and HDOA is a polymer or aggregates of 17 000–18 000 kDa as judged by HPLC (Breton et al. 1989). Such an observation has been made previously showing that heat-denatured OA aggregates exhibited an MW of 2700 kDa at 78°C; 6000 kDa at 80°C and 9700 kDa at 82°C (Kato and Takagi 1987).

These antigens induced different antibody subpopulations: as judged by affinity chromatography, anti-NOA antisera contained 87% anti-NOA specific antibodies, 7% anti-DOA specific antibodies and 8% antibodies binding both NOA and DOA. Anti-DOA antisera contained only 22% antibodies specific for DOA, 28% specific for NOA and 50% binding both NOA and DOA. However, by direct ELISA, the eight different antibody subpopulations behaved quite differently: as shown previously (Breton et al. 1989), when fixed to plastic plates, NOA was extremely denatured by interaction with the plastic, and most antibodies (Table 2) reacted with NOA on plates, even those supposed to be specific for HDOA (such as DAC₄) with the exception of NAC₃ (specific for NOA) and DAC₄ (specific for HDOA); most antibodies reacted even better against HDOA. Thus it can be concluded that direct ELISA should not be used to detect changes in antigenic properties of proteins which occurred during food processing. Indeed, on the one hand, though still of general use, direct ELISA fails to detect monoclonal antibodies, leaving these to be identified by other immunochemical tests (Friguet et al. 1984). The present observation with polyclonal antibodies emphasises the high percentage (85%) of antibodies which would not be detected by direct ELISA (Table 1). Moreover, none of the anti-HDOA antibodies was truly specific (ie recognising only HDOA) (Table 2). On the other hand, immunoprecipitation is not likely to be of use in the agrofood
industries. As we have seen, whereas NAC\textsubscript{1} was specific for NOA (Breton et al. 1989), DAC\textsubscript{1}, NAC\textsubscript{2} and DAC\textsubscript{2} were unable to precipitate NOA and were mainly directed against HDOA though not fully specific for it (Figs 2 and 4). These results were confirmed by HPLC analysis of supernates after immunoprecipitation (Figs 3 and 5).

Thus sandwich ELISA can be considered as the best technique for unambiguous identification of either NOA or HDOA, as shown by immunocapture of radioactive ovalbumin followed by sandwich ELISA (Table 3). This observation was further supported by several previous reports. For instance, by comparing seven different procedures, Al Moudallal (1984) showed that the apparent binding specificity of monoclonal antibodies depended upon the kind of ELISA test used. Direct ELISA with antigen-coated plates was the least powerful technique, but several different sandwich ELISA tests were found to be much better for specificity and sensitivity for the identification of tobacco mosaic virus. It was also shown that the specific binding of monoclonal antibodies to the native form of rat brain hexokinase was possible if the enzyme had been immobilised by polyclonal antibodies (Smith and Wilson 1986). Conversely immunocapture of a coronavirus by monoclonal antibodies increased the sensitivity of an ELISA assay (Bernard et al. 1986). In the case of ovalbumin the molecules underwent an extensive conformational change due to the adsorption of the protein on to polyvinylchloride microtitre plates, as tested with monoclonal (Kilshaw et al. 1986) or polyclonal (Breton et al. 1986) antibodies.

When used as capture antibodies, NAC\textsubscript{3} was not fully specific for NOA by immunocapture (Table 3), however, when a mouse anti-HDOA antiserum was used in a sandwich ELISA, NAC\textsubscript{3} was specific for NOA (OD = 0.4) as compared to HDOA (OD = 0), NAC\textsubscript{2} could also be used. The most likely explanation is that, due to the highly aggregated state of HDOA (MW 18 000 kDa), a NAC\textsubscript{3} molecule binds much more HDOA than NOA even though the affinity should be much lower.

Similarly, DAC\textsubscript{4} was apparently fully specific for HDOA as measured by immunocapture of radiolabelled antigens (Table 3). However, this was not the case by sandwich ELISA unless a mouse anti-HDOA antiserum was used as second antibody (Table 3). Thus sandwich ELISA using NAC\textsubscript{3} or DAC\textsubscript{4} as capture antibodies permitted identification when ovalbumin was either native or heated to below 65°C, as compared with ovalbumin heated at 85 to 100°C (Table 4). This observation holds for pure solutions of ovalbumin or canned mushroom juices with 20 g litre\textsuperscript{-1} of ovalbumin as additive.

It is becoming important to confirm adequate processing of food, particularly thermal treatment for ensuring the absence of living pathogens, most of which are destroyed by heat treatment at 75°C. However, new technologies in meat processing involve long-term heating between 56 and 85°C or short-term heating between 57 and 63°C, known as aseptic processing. The results reported above have to be related to those given by differential scanning calorimetry, which shows a steady increase of denaturation between 62 and 77°C (Hegg et al. 1979).

From these results we can speculate that a drastic change occurred during heat treatment between 65 and 85°C. If means are required to control for time/temperature processing of food, a more precise technique is needed. Thus more
specific antibodies for immunocapture have to be developed. The technique itself should be based on the assumption that, for a given protein, different conformations might be representative of different heat treatments, provided the duration of heat treatment is long enough to obtain a stabilised form of the molecule. Then only monoclonal antibodies would be specific enough to distinguish, by immunocapture, different ovalbumins that have been heated at various temperatures. Work is in progress to achieve such a goal.

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