Reviews

Immunochemical probes for food proteins after heat processing

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Summary. While better hygiene controls and vaccinations have diminished the occurrence of infectious diseases in humans, food-borne diseases have increased. Thus sterilization of food products is of prime importance. The introduction of new technologies applied to food has necessitated new methods for the control of food safety and food quality. This review aims to point out the importance of immunochemistry in the identification of structural changes induced in food proteins during food processing. New technologies have introduced the use of additives in food products, therefore it is important to identify and quantify such additives, even after complete heat denaturation. Toxic chemicals, toxins and pesticides which can contaminate food products before or during processing should also be identified. Finally, the use of immunochemical tests as a control of sterilization procedures in heterogeneous foodstuffs is discussed.

Key words. Food hygiene; food processing; immunochemistry; monoclonal antibodies.

Introduction

Most human and animal foodstuffs undergo some kind of technological processing, usually involving heat at some point in the process. Many of the important functional properties of food proteins are related to water-protein interactions such as solubility, viscosity, gelation, foaming and emulsification. The ability of proteins to bind fat is extremely important in several products, such as meat replacers and extenders, because it enhances flavor retention and improves mouth feel. The effects of partial or total denaturation on protein functionality are important in food processing: denaturation is an integral part of several processes in the preparation and texturization of foods. Simple cooking or boiling will usually unfold proteins, but unfolding also occurs during emulsion or foam formation. It can thus be inferred that the study of the fine structure of raw and processed food might give new insight into the relationship between structural modifications and food quality.

Foodstuffs are often contaminated as raw material by pathogens such as toxins, bacteria or viruses. It is important to understand the origins of such contaminations, which may vary considerably depending upon whether the foodstuffs come from developing countries or developed countries. A few examples will make this point clear. Water pollution is quite common in developing countries such as India, and as stated by Hofstra and Huis in't Veld[13] 'In water, attempts to establish international standard methods for the detection and enumeration of *E. coli* have so far failed. Most countries have their own regulations and quality standards for drinking and waste water'. As many steps of food processing involve water, it is understandable how food can be contaminated by this route. Among the most prevalent water-borne diseases in industrialized nations are hepatitis A, and gastroenteritis due to retroviruses[17]. Some viruses, such as the poliovirus[24] can be extremely resistant in sewage water, and can be found as living organisms even after 1 year of storage at either 4 °C or at room temperature.

Toxicants such as aflatoxins are produced by different strains of *Aspergillus* during food storage, and when feed contaminated with aflatoxin is consumed by dairy cattle the animal can be affected by the toxin. Furthermore, milk produced when toxic feed is consumed by the cow can contain aflatoxin, and incidents of poisoning have occurred in humans after consumption of such contaminated milk[2]. Another source of food contamination is from farm animals, which can harbor pathogens or even suffer from diseases in which there is multiplication of important pathogens such as Salmonella[22] or Listeria[21]. Surprisingly, while hygienic controls and vaccinations have led to a considerable reduction in the occurrence of infectious diseases in humans during the two last decades in developed countries, during the same period food-borne diseases have increased, as shown in figure 1 for salmonellosis[14] and in figure 2 for listeriosis[21].

In this review we shall discuss applications of immunochemistry in food quality control of different purposes:

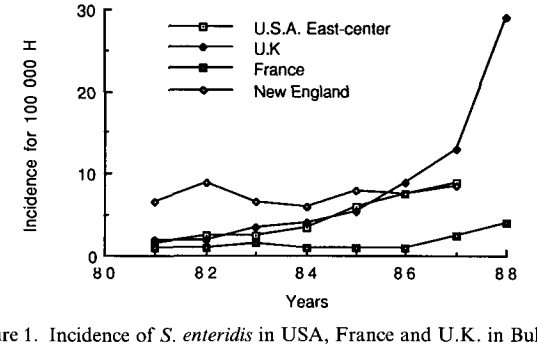

Figure 1. Incidence of *S. enteridis* in USA, France and U.K. in Bulletin d'épidémiologie B.E.A. 1989–16/89.

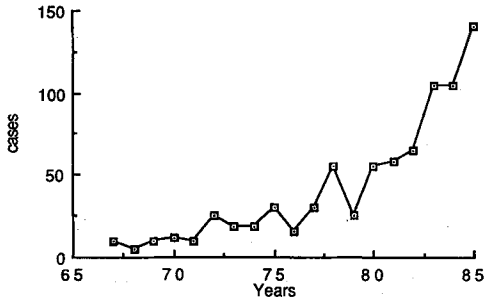

Figure 2. Numbers of cases of listeriosis per year in the U.K. where strains of *Listeria* were sent to the Division of Microbiological Reagents and Quality Control, Colindale. 1967–1975: Total 786 cases quoted in ref. 6. *Listeria*: taxonomy and epidemiology.

1) Detection of fraudulent additives; 2) Contamination by toxins and pesticides; 3) Recognition of heated proteins: checking of heating processes.

Additives in heat-processed food

As stated by A. R. Hayden[12] 'a fraudulent substitution of cheaper species of flesh or vegetable proteins for more expensive flesh in meat products is objectionable for reasons of health, religion and economics'.

Ingestion of products containing undeclared flesh, milk or vegetable proteins can induce allergic reactions in sensitized individuals. For example, β-lactoglobulin is a potent allergen in cow's milk. By using monoclonal antibodies it was found that an epitope on the molecule was modified when it was heated at 70 °C in alkaline conditions (pH 8 to pH 9); a specific monoclonal antibody against this region was a useful probe in identification of heated β-lactoglobulin[15]. However, another approach was to raise antibodies against thermostable antigens, so that antibodies raised against the native molecule could recognize heated muscle without interference due to gelatine when used as immunocaptors in a sandwich ELISA test[16]. A similar approach was used to identify soya protein in meat processed at either 100 °C for 30 min or at 121 °C for 30 min[10]; identification of soya in meat treated at 100 °C was quantitative from 2 to 32%, while in beefburger treated at 121 °C the slope was still linear but with a constant reduction.

Other adverse effects have been observed in humans consuming soybeans which contain inhibitors of proteolytic enzymes, which adversely affect nutritional quality and safety[18]. Since heat treatments used commercially only partially inactivate trypsin inhibitor activity, polyclonal and monoclonal antibodies were raised against soybean trypsin inhibitors[4]. Antibodies elicited with the heat-denatured inhibitors were specific for the denatured conformation of the protein; in contrast, native inhibitors induced antibodies that selectively recognized determinants in both native and heat-treated proteins.

Foreign proteins are sometimes added with the fraudulent purpose of either making the food product less expensive to produce or to add a very cheap additive to a very expensive food. We shall report two examples of such frauds:

Ovalbumin added to increase the apparent weight of canned mushrooms

Canned mushrooms are widely used in Europe to mix with sauces or to accompany meat or fish dishes. A large proportion of these mushrooms are raised in the Loire Valley, and they are used in French factories or are exported as a fresh product mainly to Holland and Germany where they are processed. A few years ago, French producers of canned mushrooms observed a drastic drop in their exports due to the fact that canned mushrooms from either Holland or Germany were sold at a 10% discount for cans in which mushrooms weighed 10% more than those produced in France after draining. To make a long story short: cultivated mushrooms possess a 'foot' in which large tubes can be filled up by water, which will stay in the tubes if the mushrooms are heated in a 5% egg-white solution at 95 °C. Thus ovalbumin coagulation will make a deposit on the surface of the mushrooms, preventing the water from draining away.

During our studies we faced two difficulties: protein extraction from mushrooms heated at 130 °C and quantification of the foreign protein. Protein extraction of coagulated protein required solubilization of both ovalbumin and mushroom proteins. Protein solubilization can be quantitated by comparison of absorbance at 280 nm between native and solubilized heat-denatured proteins. In the case of pure ovalbumin (table 1)[5] it can be seen that

Table 1. Solubilization of coagulated ovalbumin[a]

Solubilizing agents	Percent of solubilization
0.1 N NaOH	98
0.05 N NaOH	100
0.01 N NaOH	25
0.001 N NaOH	4
PBS + SDS 1%	100

[a] Ovalbumin solutions (1% in PBS) were heated at 100 °C for 30 min. Coagula were resuspended either in PBS + SDS 1% for a few minutes or in NaOH at different molarities overnight at 4 °C. Tubes were then centrifuged at 10,000 × g for 5 min and protein was determined in the supernatants[5].

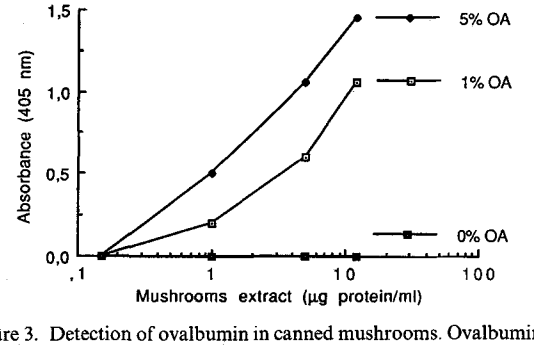

Figure 3. Detection of ovalbumin in canned mushrooms. Ovalbumin has been added to canned mushrooms before processing, cans were heated for 30 min at 130 °C, then ovalbumin has been extracted and has been revealed in an indirect ELISA.

Table 2. Quantification of ovalbumin in canned mushrooms[6]

	Limit of antigen detection[c]	µg Ovalbumin/g of drained mushrooms	% Ovalbumin in 'blanching' solution
Experimental[a]	70	50 ± 3	1
Experimental	200	200 ± 18	5
Experimental	0	0	0
Holland[b]	640	472 ± 50	10[d]
Holland	40	27 ± 4	0.5
Holland	400	329 ± 27	8
Belgium	80	49 ± 4	1
Belgium	100	109 ± 12	2
France	120	116 ± 9	2
France	100	92 ± 8	2
France	160	137 ± 15	3

[a] Canned mushrooms treated with known amounts of ovalbumin. Results are the mean of at least three determinations. [b] Commercial canned mushrooms obtained from supermarkets in Holland, Belgium and France. Among 50 cans, 24 exhibited a positive reaction. [c] Results are expressed as the lowest dilution of mushroom extract giving an absorbance value of 0.5 at 405 nm in quantitative ELISA test. [d] Estimated values from results for experimental cans.

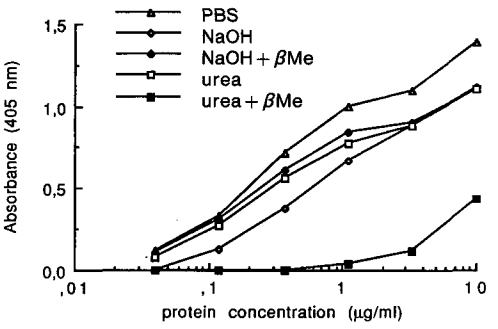

Figure 4. Ovalbumin extraction from 'foie gras' by five different methods as measured in an sandwich ELISA.

solubilization of a 1% coagulated ovalbumin was obtained either by 0.1 N NaOH or by PBS + 1% SDS with the same yield. However, it appeared that SDS could destroy some epitopes of the molecule (results not shown), so we used the alkaline treatment which was required to reach a 98% yield of protein extraction. Twenty minutes were sufficient to detect the maximum yield of ovalbumin from canned mushrooms at 4 °C under constant agitation.

Quantitative analysis of ovalbumin in mushroom extracts was carried out by ELISA with anti-native antibodies purified by affinity chromatography on an ovalbumin (OA) column[6]. With experimental cans (fig. 3), a minimum of 1 µg protein/ml in mushroom extracts was found to be sufficient to detect ovalbumin when present and it was possible to differentiate 1% from 5% ovalbumin. Table 2 shows the results of examining canned mushrooms[6]. The same range of weight OA/weight mushrooms was found as in the experimental samples: based on the ovalbumin content measured by direct ELISA, calculations showed that 1–10% ovalbumin had been added in these commercial products.

A fine analysis of specificities found among polyclonal antibodies[7] showed that by affinity chromatography antibodies specific for either OA or HDOA, NAC_3 and DAC_4, respectively, could be separated. Moreover, when a sandwich ELISA was performed instead of the indirect ELISA the critical step was the antigen immunocapture, and this observation holds for monoclonal antibodies as well.

Ovalbumin added to 'foie gras'

'Foie gras' is supposed to be made of pure goose liver with goose fat and any additive is forbidden. However, defects can arise due to an excess of fatty liver with a bad texture, depending upon the quality of the goose. In order to improve the texture quality of either 'foie gras' or 'pâté de foie gras' it was found that addition of ovalbumin up to 10% of total protein could improve both products. From the economic point of view it is very interesting for the producer; from the hygienic point of view, some trouble can occur in allergic consumers.

'Foie gras' and 'pâté de foie gras', sold as preserved foodstuffs, are heated at either 62 °C, 85 °C or 105 °C and contain up to 70% fat, which makes protein extraction difficult. Six methods were applied to dissolve 'foie gras' heated at 62 °C: PBS, PBS + NaOH 0.1 N, PBS + NaOH 0.1 N + βMe 0.2%, urea 8 M and urea 8 M + βMe 0.2%. As seen in figure 4, for heated 'foie gras' at 62 °C, all methods but the last one could be applied: however, extraction in PBS was found to be a little more sensitive than other methods, with a sensitivity of 100 µg of total protein containing amounts of ovalbumin as low as 1%. Sensitivity increased after dialysis of protein extracts.

Toxins and pesticides in contaminated food

The origins of food contaminants can be multiple. At one point in processing, water is often used, and this can bring bacteria or viruses which can be identified by specific polyclonal or monoclonal antibodies. The main difficulty in most cases is the necessity of enriching the medium by culture, or sometimes by inoculation into experimental animals, which increases considerably the period which elapses between the time of harvesting of the food and time when results of tests for hygienic to quality are available. Most bacterial and viral contamination comes from animal food, mainly from meat, but also from milk. Due to the heat-stability of staphylococcal enterotoxins and the relative frequency of poisoning incidents caused by *Staphylococcus aureus* enterotoxins, it is important not only to identify bacteria in contaminated meat, but also to characterize specific toxins. Different methods for doing this are reviewed by Smith and Bencivengo[27]. For food quality control a detection method should be able to detect 0.125–0.250 µg of enterotoxin per 100 g of food[25]: by an ELISA method with

Table 3. Comparison in sensitivity of RIA and ELISA tests[9]

Mycotoxins	Standard ranges		Detection limits µg/kg	
	RIA	ELISA	RIA	ELISA
AFB_1	500–5000	25–1000	5.8	3
AFM_1	5000–50,000	25–1000	5	0.5
T_2	200–2500	2.5–200	1	2.5
Zearalenone	250–10,000			

monoclonal antibodies it was possible to detect 1 µg of enterotoxin per 100 g of sausage, and a polyclonal serum used in an ELISA test was also enough to give sufficiently reliable yes/no answers.

Methods for the detection of fungi in food include culture methods and direct microscopy, and also immunochemical methods. The latter were shown to be extremely reliable for the detection of molds in different foods such as tomato purée, peach purée, apricot nectar, applesauce, fresh bread and cottage cheese[19]. The formation of mycotoxins in foods occurs under conditions of humidity where fermentative processes can take place in vegetables and plants. The occurrence of mycotoxins has an important impact on humans and animals, in particular in developing countries, and also in developed countries when food has not been properly stored. Interest in the use of ELISA techniques for the detection of low doses of mycotoxins (in the range from 0.1 µg/l in milk to 5 µg/kg in peanuts) has been reviewed[9]. Comparison between the sensitivities of RIA and ELISA showed an equal sensitivity (table 3).

Aflatoxins are toxic and carcinogenic secondary metabolites produced by some common Aspergilli during growth on animal feeds or foods, and thus it is important to identify the type of mycotoxin produced. AFM_1 is among the aflatoxins most frequently found in the dairy industry, as shown in table 4. This is due to the fact that when AFM_1 is present in raw milk, where it appears to be associated with the casein fraction[2], cheese manufacture will concentrate AFM_1. However, as mentioned by Applebaum et al.[2] sterilization at 70–75 °C will reduce the amount by 10–40%, and at 120 °C by 25–80%.

When quantitative assays are required the following steps should be performed, as described by Applebaum et al.[2]. 1) Sampling; 2) Extraction of the aflatoxins; 3) Clean-up procedure to eliminate lipids and interfering substances; 4) Separation; 5) Quantification. Extraction was shown to be a critical step for the recovery of aflatoxin, and HPLC, though costly, is probably the best technique. ELISA does not need extraction or clean-up, and kits for the test are now on the market.

The use of immunochemical methods of analysis for environmental chemistry is recent[11]. The method should be applied to a variety of compounds difficult to analyze by other methods. For example, poisoning by pesticides can be identified by applying immunochemistry to urine or blood from patients.

Pesticides applied to a field may contaminate either the water which will be used for food preparation or the plants themselves. Contaminated food or plants will in turn contaminate man either directly, or indirectly by contamination of animals feeding on the plants (pesticides will then be found in milk and meat). One concern is that geneticists have developed plants resistant to herbicides, allowing the use of higher concentrations of pesticides, which will increase food contamination risks.

Another aspect of toxicity is the allergies induced in man by many compounds found in milk, such as bovine β-lactoglobulin[15], or in meat additives containing grain products such as wheat, rye, barley or oats, which contain gluten. The effects of heating or baking on gluten detection by antibodies were studied by Skerritt[26]. They showed a 95% loss of immunoreactivity from extracts heated for 10 min at 100 °C, with a limit of sensitivity of 20 µg/ml of extract. We conclude that antibodies specific for heated gluten are required.

Recognition of heat-processed food: Effects of conditions of heating processes

Fraudulent substitution of cheaper species of flesh or vegetable proteins for more expensive flesh in meat or seafood has led to an increasing demand for means to identify such additives. However, in most cases foods are heated, for technological or hygienic reasons, so that the protein structure can be considerably modified, altering not only immunochemical reactions[12], but also electrophoretic patterns[1], behavior in HPLC[20], enzymatic activity[28] and other properties, as reviewed by Townsend and Blankenship[29]. From the immunochemical point of view it can be assumed that depending upon the time/temperature ratios used for heat processing of foods, protein structure will be modified and thus the immunochemical approach should be to design antibodies specific for epitopes which appear or disappear during the heating process. Structural modifications of proteins will be more pronounced in large protein molecules

Table 4. Aflatoxin M_1 content of various commercial cheeses collected in Germany[23]

Cheese	Number of samples tested	% Positive	AFM_1 concentration (µg/kg) Min	Max	Ave
Fresh cheese	80	34	0.10	0.51	0.23
Camembert	65	51	0.10	0.73	0.31
Hard cheese	77	75	0.10	1.30	0.43
Farmer's cheese	134	40	0.10	0.55	0.26

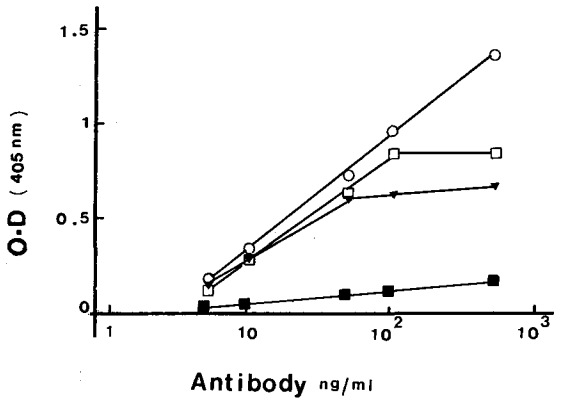

Figure 5. Antibody binding of native or heat-treated ovalbumin as measured in an indirect ELISA: native ovalbumin, OA (■); non coagulated, heat-treated ovalbumin, HDOA NC (▼); coagulated ovalbumin solubilized in 0.1 N NaOH, HDOA-NaOH (○) or in PBS-1% SDS, HDOA SDS (□). The antigen concentration was 0.1 μg/ml.

(mol.wt > 20,000) than in small protein molecules, as it has been shown that the number of epitopes was partly correlated with the length of the molecule; for instance while the c-type lysozyme (mol.wt = 14,000) exhibited 3 or 4 epitopes[3], human serum albumin (mol.wt = 67,000) expressed 7 or 8 epitopes[8]. Such changes in the availability of epitopes will depend on the environment of the molecule, including pH, and the presence of salts, foreign proteins and sugars, and on the homogeneity of the product. Such structural modifications are easier to control in protein solutions than in solid or semi-solid foods, for which a solubilization step is required before an immunochemical method can be applied. During the solubilization procedure new conformations could arise, resulting in changes in the epitopic expression.

The origin of antibodies used for immunochemical tests such as the ELISA test can be misleading; we found, for instance, that using a direct ELISA (with the antigen bound to a microplate), antibodies from rabbits immunized against the native ovalbumin bound the heat-denatured ovalbumin more strongly than the native ovalbumin (fig. 5), whether the antigen had to be solubilized or not[5]. In fact, in the anti-native ovalbumin serum antibodies specific for the heat-denatured ovalbumin were present and could be affinity purified (NAC_4) (fig. 6) besides specific anti-native ovalbumin antibodies (NAC_3). Moreover, depending upon the type of ELISA

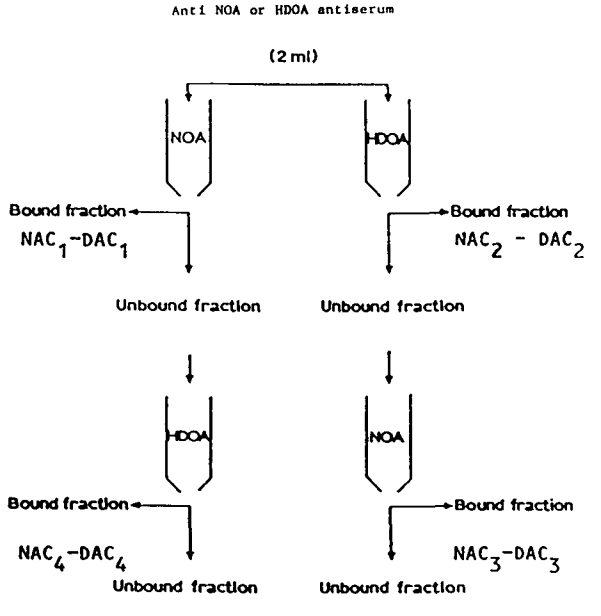

Figure 6. Affinity purification of rabbit anti-NOA (NAC_1-NAC_4) or anti-HOAD (DAC_1-DAC_4) antibodies.

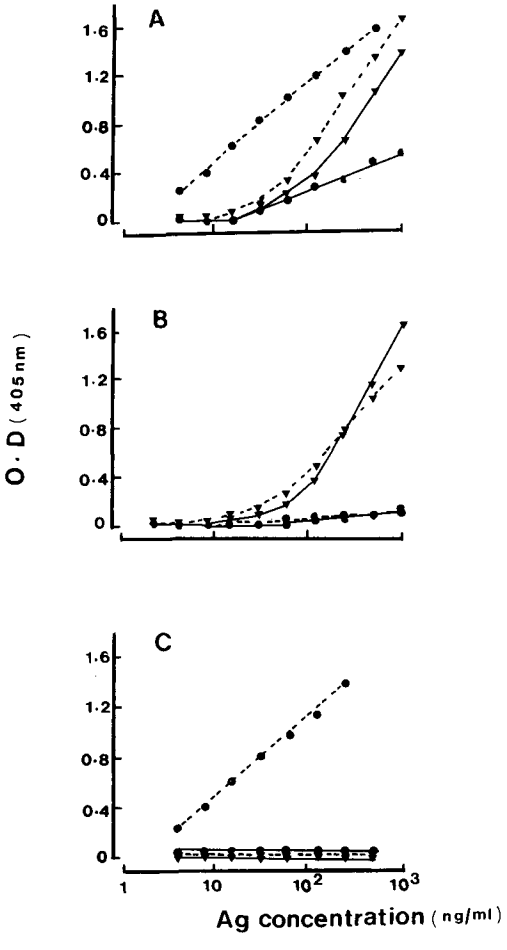

Figure 7. Comparison between indirect (——) and double antibody sandwich (– –) ELISA for the detection of NOA (●) and HDOA (▼) with Ac_1 (A), Ac_2 (B) and Ac_3 (C) antibodies coupled to alkaline phosphatase. In the indirect ELISA test, NOA and HDOA were absorbed directly to the solid phase. In the double antibody sandwich ELISA test, NOA and HDOA were bound to rabbit anti-NOA antibodies absorbed to the solid phase (Breton et al.)[7].

Table 5. Recognition of heat processing by polyclonal antibodies

Antibodies used for immunocapture[2]	Untreated[1]	Heat treatments 50 °C[1]	65 °C[1]	85 °C[1]	100 °C[1]
NAC_3	0.500[3]	0.500	0.500	0	0
DAC_4	0	0	0	0.700	0.700

[1] Antigen revealed by mouse polyclonal antibodies; [2] Affinity purified rabbit antibodies; [3] Absorbance: O.D.

used, different conclusions can be drawn [7]: when an antiserum was partially purified (NAC_1), direct ELISA discriminated much less clearly between native ovalbumin and heat-denatured ovalbumin than did completely purified antibodies (NAC_3), which bound native ovalbumin specifically when a sandwich ELISA was performed (while no antigen-antibody interaction was detectable in direct ELISA) (fig. 7).

In conclusion, in order to increase the specific recognition of heat-denatured ovalbumin and native ovalbumin, different affinity purified antibodies should be used in a sandwich ELISA test. With NAC_3 as immunocaptor, only native ovalbumin and ovalbumin heated to either 50 °C or 65 °C were identified, whereas with DAC_4 as immunocaptor, only ovalbumin heated at either 85 °C or 100 °C was recognized (table 5). It can be concluded that a drastic change in ovalbumin structure occurred when the solution was heated between 65 °C and 85 °C.

We expected that using monoclonal antibodies for immunocapture would be more discriminant than using polyclonal antibodies. Thus 10 µg/ml samples of ovalbumin in a saline solution were heated for 10 min at the following temperatures: 65 °C, 70 °C, 75 °C, 80 °C and 85 °C. We prepared more than 20 different monoclonal antibodies by immunizing mice either with native ovalbumin or with heat-denatured ovalbumin (100 °C). Monoclonal antibodies in ascitic fluids were prepared by injecting cell-lines intraperitoneally in pristane-sensitized mice: harvested ascitic fluid was then precipitated by ammonium sulphate in order to partially purify immunoglobulins. Preparations with 10 mg/ml in PBS were stored in the cold room. The monoclonal antibodies were coated on plastic plates, then antigen solution at 100 ng/ml was added and its immunocapture was revealed by rabbit polyvalent polyclonal antiserum. In table 6 it can be seen that Mabs 1 and 2 gave a strong reaction with the whole range of proteins, from native up to that heated at 75 °C, and only weak recognition of ovalbumin heated at 80 °C. Conversely, Mabs 3 and 4 exhibited a weak reaction with protein heated at 75 °C and a strong reaction with that heated at either 80 °C or 85 °C.

However, in order to ascertain the conditions to which a protein was exposed during the heating process, not only the maximum temperature of the process should be identified but also the time during which this temperature was reached. Thus the same ovalbumin solution was heated under the conditions described above for different times; 1, 3, 9 and 30 min and immunocapture of the antigen was performed with either 50 ng or 10 ng/ml of ovalbumin. As shown in figure 8, with the monoclonal antibody KA_4B_4, heating of the ovalbumin at 70 °C for either 1, 3, 9 or 30 min did not modify the optical density, whatever concentration of antigen was used in the ELISA test. On the other hand, large modifications were seen for ovalbumin heated at either 75 °C or 80 °C. From this result it can be concluded: 1) A 5° difference in the heating process can be identified by only one Mab. 2) The antigen concentration used for immunocapture plays an important role: for instance if ovalbumin has been heated at 75 °C a 10 ng/ml solution gives a 50% inhibition if the heating procedure has been applied for 9 min; in contrast, using a 50 ng/ml solution there was no inhibition even for the ovalbumin solution heated for the same time. It can be seen in figure 9 that if ovalbumin has been heated at 80 °C the time that elapses during the heating process is detected only if the antigen solution contains either 4 or 12 ng/ml. The conclusion is that the KA_4B_4 epitope was lost in 1 min, which correlates with the test made with 10 ng/ml, as shown in figure 8. 3) This

Table 6. Recognition of heat processing of an ovalbumin solution in a sandwich ELISA test

Monoclonal antibodies[1] Mabs	Native OA	Heat treatments 65 °C	70 °C	75 °C	80 °C	85 °C
1 KA_4B_4	1060	950	850	850	200	0
2 QA_6B_5	1650	1550	1480	1400	220	0
3 BC_4F_2	0	0	0	310	650	650
4 IB_4E_5	0	0	0	390	780	790
5 AD_6C_4	1600	1600	1600	1500	800	200
6 IA_2A_2	630	600	600	570	100	0
7 $G_1C_6D_{11}$	0	0	0	0	450	600
8 $S_7B_4E_9$	0	0	0	100	580	700

[1] Revealing antibodies: rabbit NAC_2.

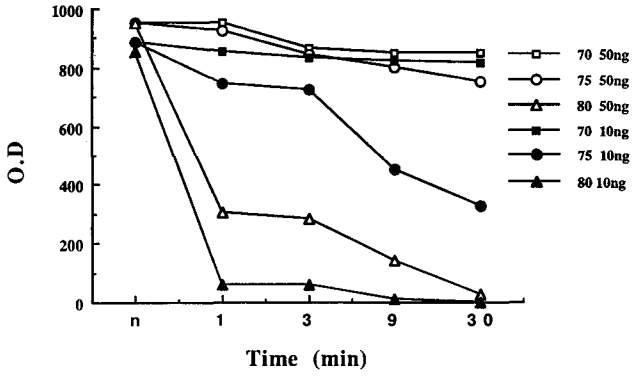

Figure 8. Ovalbumin structure modifications upon different heating processes as measured by the monoclonal antibody KA_4B_4 which was used as a captor in an ELISA sandwich.

epitope is drastically changed in 9 min when the heating process has been performed at 75 °C, or in 1 min when the heating process has been performed at 80 °C.

Conclusion

Immunochemical methods are extremely versatile, and are characterized by: 1) high specificity; 2) high sensitivity (from ng to µg for 1 g or 1 ml of product); 3) rapidity.

As shown in table 7, by the use of antibodies one can solve many problems in order to check food quality and food safety and also to check processes in food technology.

Heating processes are performed either to improve food quality or to ensure food safety by killing pathogens such as viruses, bacteria or parasites. For immunochemical tests on food products, the proteins need to be solubilized: thus while these techniques can be applied directly to liquid foods such as milk, beer or wine, they require a solubilization step when solid food such as meat, or a cereal product is under study. During solubilization procedures, it may happen that either the target protein cannot be solubilized, or epitopes might be modified, making immunochemistry difficult to apply. For instance during heating procedures ovalbumin will be coagulated and many epitopes present on the molecule will disappear; by using NaOH as a solubilizing agent, and polyclonal or monoclonal antibodies specific for the heat-denatured form of ovalbumin, it is however possible to apply ELISA tests. In order to identify the presence or absence of food components qualitatively, these techniques will not be difficult to apply; however, quantitative assays will require a complete solubilization, which in the case of food very rich in fat might be difficult to perform as in the case of 'foie gras'. In many cases, only a qualitative assay is required, and partial solubilization has been shown to be sufficient, for instance to characterize a fraudulent additive.

One of the most interesting applications of immunoassays will be the checking of heating procedures. Accurate techniques have been designed for following heating processes, for instance more and more sophisticated thermocouple probes can be introduced in the food such as milk or meat. However, there is still the drawback that the product may be heterogeneous, with 'cold points' in small areas where living organisms could survive. By harvesting and solubilizing samples from a large enough area, immunochemical tests could be used for identification of protein structures related to the minimum temperature reached at these cold points. As each protein will express different epitopes, depending upon its origin, on neighboring proteins, on the distribution of fat and water, and finally on the temperature at which the food has been heated, it will be necessary to raise antibodies specific for each type of food.

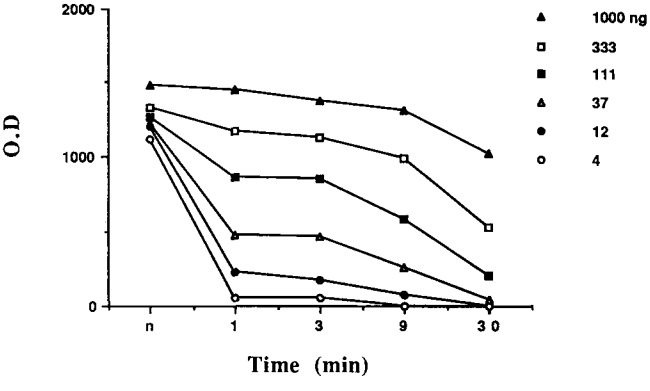

Figure 9. Role of the antigen doses in an ELISA test to identify the heating time for ovalbumin heated at 80 °C, revealed as in fig. 8.

Table 7. Use of antibodies in the food industry

1) *Raw material identification*
   a) Bacteria: Typing for fermentations — Surface proteins / Enzymes
   b) Plants and grains: Species and Subspecies — Reserve proteins / Enzymes
   c) Animals: Muscles, Organs
2) *Detection of contaminants in raw material*
   a) Pesticides
   b) Toxic substances
   c) Viruses, bacteria, parasites
3) *Identification of components and contaminants in processed foods*
   a) Regular additives: quantitation
   b) Fraudulent additives: plant and grain species and subspecies, meat or fish of different origins
   c) Toxins: mycotoxins, staphylotoxins allergens, pesticides
   d) Viruses, bacteria, parasites
4) *Control of food processing*
   a) On line: fermentation: optimization
   b) After processing: control of end products, heating processes, fermentative processes

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