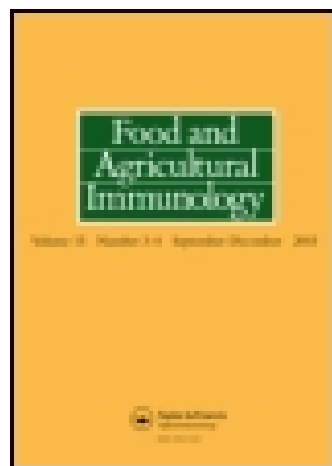




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An Immunoassay for Distinguishing between Crustacean Tailmeat and White Fish

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An immunoassay has been developed which facilitates discrimination between white fish, such as coley, and crustacean tailmeat, such as scampi. Preliminary studies with this non-competitive indirect ELISA clearly demonstrate the feasibility of using immunochemical tests for detecting adulteration of high value crustacean tailmeat products with lower value white fish.

Keywords: *Adulteration, Authenticity, ELISA.*

INTRODUCTION

Fish mince, produced by the mechanical de-boning of low-cost fish, provides a versatile and protein-rich raw material with which to develop value-added products for human consumption (Venugopal, 1992). However, such materials also provide scope for the wilful or unintentional adulteration of high-quality, expensive seafood products with cheaper fish meats. For example, the production of reformed, bread-crumbed scampi pieces—which makes up approximately 90% of the UK scampi market (Deeprise, 1991)—involves reforming chopped scampi pieces prior to extrusion, breading and flash frying. Clearly there is potential for adulteration of such products with cheaper white fish.

An obvious approach to detecting such adulteration is the development of immunochemical methods for distinguishing between meats originating from fish and crustacea. Antisera raised against water-soluble extracts of canned sardine, albacore tuna, yellowfin tuna, skipjack tuna and bonito, during the development of immunochemical methods for authenticity testing of these products, cross-react with a range of sardine-type and tuna-type fish—both canned and raw (Taylor *et al.*, 1992). In order to assess the feasibility of developing an immunochemical test to discriminate between white fish and crustacean tailmeat, the relative immunoreactivities of these antisera with extracts of coley, cod, haddock, scampi and prawn were determined in a non-competitive indirect ELISA.

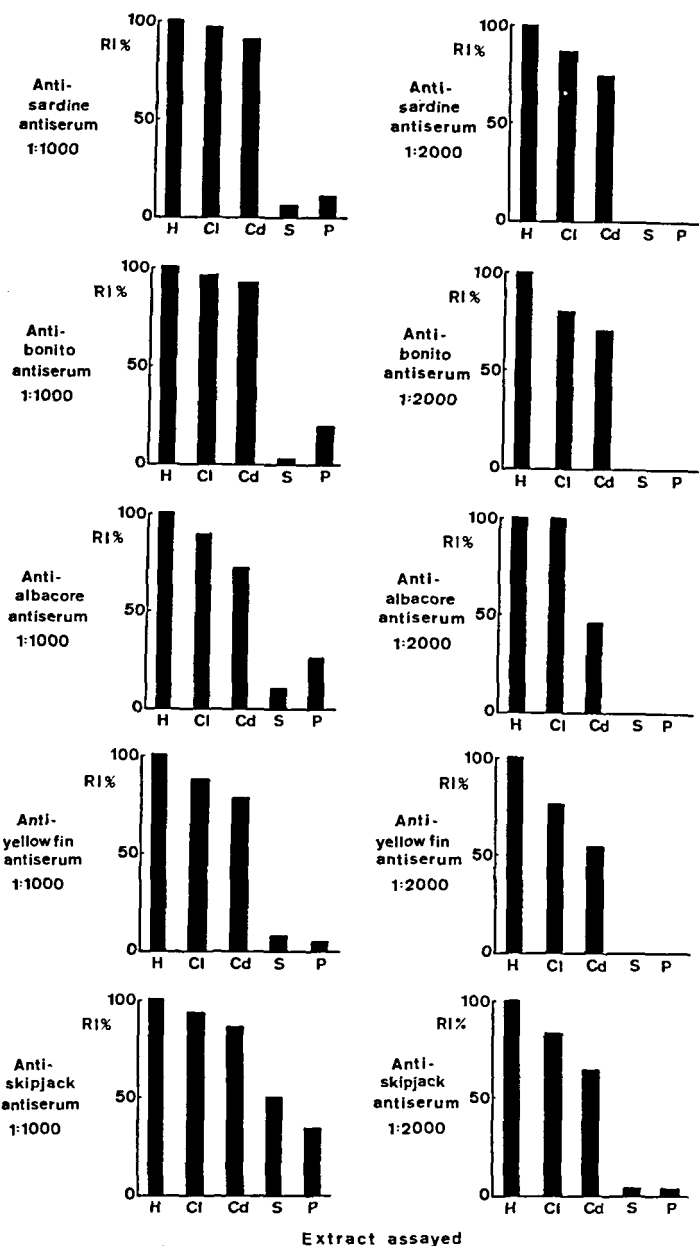


FIG. 1. Relative immunoreactivities of five antisera with extracts of white fish and crustacean tailmeat. Antisera (at 1:1000 and 1:2000 dilutions) were assessed in a non-competitive indirect ELISA against extracts of haddock (H), coley (Cl), cod (Cd), scampi (S) and prawn (P). Relative immunoreactivities (RI%) were calculated for each antiserum by taking the OD obtained against haddock extract as 100% and expressing the ODs obtained for the other extracts as percentages of this. Typical signal intensities obtained with the antisera (1:1000) against haddock are 1.09 (anti-sardine), 0.96 (anti-bonito), 0.99 (anti-albacore), 0.85 (anti-yellowfin) and 1.23 (anti-skipjack).

MATERIALS AND METHODS

Antiserum Production

Water-soluble extracts of canned preparations of sardine (*Sardina pilchardus*), bonito (*Sarda orientalis*), albacore tuna (*Thunnus alalunga*), yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Euthynnus* spp.), and rabbit polyclonal antisera against these extracts, were produced as described previously (Taylor *et al.*, 1992; Taylor & Jones, 1992).

Non-competitive Indirect ELISA

A Dynatech 96-well polystyrene microtitre plate was coated with the water-soluble extracts of cod (*Gadus morhua*), coley (*Pollachius virens*), haddock (*Melanogrammus aeglefinus*), scampi (*Nephrops norvegicus*) and prawns (whole crustacean or tails of species of Palaemonidae, Penaeidae and Pandalidae), which were prepared as for sardine and tuna. The ELISA was carried out as described previously (Taylor & Jones, 1992) using 50 μ l of anti-sardine, anti-albacore, anti-bonito, anti-skipjack or anti-yellowfin antisera (diluted 1:1000 or 1:2000 in 1 mM-phosphate-buffered saline, pH 7.4) as primary antisera, alkaline phosphatase-labelled goat anti-rabbit IgG (Sigma Chemical Company, Poole, UK) as detection antibody and *p*-nitrophenyl phosphate (Sigma) in diethanolamine buffer (pH 9.8) as the substrate.

The percentage relative immunoreactivity (*RI*%) of the five different antisera with each fish and crustacean extract was calculated by taking the mean OD (405 nm) exhibited by the antisera in wells coated with haddock as 100%, and calculating the mean OD obtained in wells coated with other extracts as a percentage of this.

RESULTS AND DISCUSSION

The results are presented graphically in Figure 1. Clearly, the antisera show a much greater immunoreactivity with the white fish than with the crustacean tailmeat. For example, at 1:1000 dilution, the anti-sardine antibody reacts relatively strongly with white fish (e.g. haddock 100%, cod 92%) but much less so with extracts of crustacean (e.g. prawn 12%, scampi 6%). At this dilution the anti-bonito, anti-albacore and anti-yellowfin antisera gave broadly similar results, though the anti-skipjack antiserum showed a higher relative immunoreactivity with the extracts of scampi (51%) and prawn (35%).

When the antisera are diluted further, the differential between immunoreactivities obtained with fish and crustacean extracts becomes greater (Figure 1). For example, at 1:1000 dilution, the anti-bonito antiserum reacts strongly with white fish meat extracts (e.g. from 93% with cod to 100% with haddock) and slightly with crustacean meat extracts (3% with scampi and 20% with prawn). However, at 1:2000 dilution the anti-bonito antiserum still reacts significantly with white fish meat extracts (e.g. from 72% with cod to 100% with haddock) but shows zero immunoreactivity with the crustacean meat extracts. Similarly, at this dilution the anti-sardine, anti-albacore and anti-yellowfin antisera give zero immunoreactivity with the extracts of scampi and prawn whilst still giving high relative immunoreactivities with the white fish extracts. At a dilution of 1:2000, even the anti-skipjack antiserum gives relative immunoreactivities of less than 3% with the scampi and prawn.

Taken together, these and previous data (Taylor *et al.*, 1992; Taylor & Jones 1992) clearly demonstrate that it is possible to produce antisera which exhibit relatively high cross-reactivities with a wide range of fish extracts and relatively low cross-reactivities with crustacean tailmeat extracts. This, in turn, demonstrates the feasibility of developing an immunochemical test for detecting the presence of fish in high-value crustacean tailmeat products. Evidently the approach offers a potentially powerful tool with which to detect possible adulteration of such high-value products.

A significant amount of work remains to be done before such a method could be used routinely to ensure, with confidence, the authenticity of high value crustacean tailmeat products. The antisera used in these experiments were raised against canned sardine and canned tuna, and not against those white fish or crustacean extracts (e.g. prawn waste) more likely to be used in adulteration of high value crustacean tailmeat products. However, it is possible that antisera raised against these fish or crustacean antigens could facilitate even better discrimination between scampi and potential adulterants. Furthermore, the commercial success of meat speciation tests (Goodwin, 1992) suggests that rapid test formats could be developed to provide a simple, effective and flexible means of detecting adulteration of fish and crustacean products at the points of manufacture and sale.

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