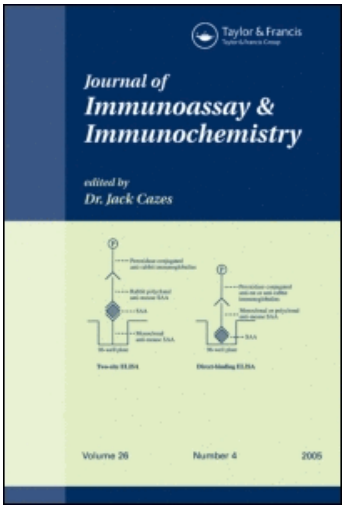


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# Differentiation of Gelatins Using Polyclonal Antibodies Raised Against Tyrosylated Bovine and Porcine Gelatins

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# Differentiation of Gelatins Using Polyclonal Antibodies Raised Against Tyrosylated Bovine and Porcine Gelatins

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**Abstract:** Gelatin is obtained from bones and hides/skin, mainly from cows and pigs using alkaline or acidic processes. The use of bovine gelatin in feed, food, and pharmaceutical products has been restricted by regulatory authorities as a consequence of the outbreak of bovine spongiform encephalopathy (BSE). On the other hand, some religions ban the porcine gelatin for human consumption. Thus, there is a need for methods able to control the species origin of gelatins. The large similarity in structure of gelatins from different origins has made unsuccessful their differentiation by physicochemical methods. Moreover, the development of immunochemical methods has been hampered by the poor immunogenicity of gelatins.

We obtained high titers antibodies upon immunization of rabbits with tyrosylated bovine and porcine gelatins. Using indirect and competitive indirect ELISAs we observed large differences in titers and specificity among rabbits and during the course of immunization. Some of the antisera were not sensitive to the species origin of raw material or to the process used for gelatin production and could be used for gelatin quantitation in food. Other antisera detected the porcine acidic gelatins with 10- to 30-fold higher sensitivity than their bovine counterparts and could be used for the differentiation of the species origin of gelatins.

Lastly, other antisera were highly sensitive to subtle changes in conformation of gelatins obtained by alkaline or acidic processes such as a 1,000-fold higher reactivity of bovine acid hide gelatin compared to that of its limed counterpart or a 30,000-fold higher reactivity of porcine acid bone compared to that of its limed

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counterpart; such antisera could be used to monitor the process induced structural changes of collagen during its transformation to gelatin.

**Keywords:** Gelatin, Polyclonal antibodies, Tyrosylated bovine and porcine gelatin



## INTRODUCTION

Gelatin is a traditional functional protein having the ability of forming transparent gels under specific conditions. It is generally obtained by heat dissolution at alkaline or acidic pH and partial hydrolysis of collagen contained in bones and hide/skin, mainly from cows and pigs. Gelatin is largely used in the food industry, especially in desserts, candies, bakery products, jellied meal, ice cream, dairy, and meat products. Gelatin is also used for manufacture of pharmaceutical capsules, ointments, cosmetics, tablet coating, emulsions, and plasma substitute. Gelatin also finds application in photography and some specialized industries.[1]

As a consequence of the outbreak of bovine spongiform encephalopathy (BSE) in Europe, the Regulatory Authorities have made restrictions to the use of bovine gelatin for human consumption, cosmetic, and pharmaceutical products. In other respects, some religions ban the use of porcine gelatin for human consumption. Therefore, it became necessary to develop analytical methods intended to control the species origin of gelatin.

Physicochemical methods based on principal component analysis of amino acid residues obtained after acid hydrolysis[2] or calcium phosphate precipitation test[3] have been unsuccessful to detect mixtures of bovine gelatin in porcine gelatin. Immunochemical methods have been proven useful to control the species origin of animal tissues in food[4,5] and pharmaceutical products.[6–8] Hofmann et al.[9] have tested an ELISA directed against a heat stable muscle antigen. The test was influenced by gelatin type and quality and the authors concluded that this ELISA cannot be reliably used for species identification of commercial gelatins.

Development of immunochemical analysis of gelatins has been probably hampered by the well known weak immunogenicity of this protein. However, Sela and Arnon[10] have demonstrated that the enrichment with tyrosine converted gelatin into a relatively powerful antigen. Our aim was, thus, to develop species-specific antibodies by rabbit immunization against tyrosylated bovine and porcine gelatins. We demonstrated that the titer and species specificity of antibodies largely varied among rabbits and during the course of the immunization. Moreover, some of the antisera appeared unexpectedly highly sensitive to the alkaline or acidic process used for gelatin production. Other antisera were quite insensitive to the species or process origin of the gelatin. Thus, a careful monitoring of the antibody response during rabbit immunization against tyrosylated gelatins is needed in order to select the antiserum adapted to the required use.

## EXPERIMENTAL

### Chemicals

Freund's complete and incomplete adjuvants, human serum albumin (HSA), N-acetyl-L-tyrosine, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimidehydrochloride (EDAC), N-hydroxysuccinimide (NHS), 2-mercapto-ethanol, hydroxylamine, gelatin type A from pig skin (300 Bloom), and type B from bovine skin (75 Bloom) were purchased from Sigma Chemical Company (St Louis, MO, USA).

Samples of industrial batches of bovine and porcine gelatins were kindly donated by Rousselot (L'Isle sur la Sorgue, France). Their characteristics are described in Table 1.

Peroxidase labelled goat IgG against rabbit IgG was purchased from Nordic (Tebu, 78510 Le Perray en Yvelines, France) and Sure Blue TMB from KPL (Thermolab systems, BP249, 95615 Cergy-Pontoise, France).



### Tyrosylation of Bovine and Porcine Gelatins

Tyrosine was activated by adding 30 mM EDAC and 60 mM NHS to a 300 mM solution of acetylated tyrosine in 10 mM phosphate buffer pH 6.0. The mixture was incubated 15 min at room temperature and EDAC was then quenched by adding 0.05 mL 2-mercapto-ethanol. The activated tyrosine was diluted 3 to 30 mM in 10 mM phosphate buffer pH 6.0.

***Table 1.*** Characteristics of the industrial batches of gelatins

| Gelatin no. | Species | Tissue | Process | Viscosity (mPa · s) | pI | Bloom | |
|---|---|---|---|---|---|---|---|
| 1 | Bovine | Bone | Acid | 3.20 | 7.3 | High | 231 |
| 2 | Bovine | Bone | Acid | 1.50 | 6.6 | Low | 50 |
| 3 | Bovine | Bone | Lime | 4.54 | 4.9 | High | 274 |
| 4 | Bovine | Bone | Lime | 2.10 | 4.9 | Low | 93 |
| 5 | Bovine | Hide | Sodic | 4.87 | 5.0 | High | 206 |
| 6 | Bovine | Hide | Sodic | 1.60 | 4.9 | Low | 40 |
| 7 | Bovine | Hide | Acid | 3.30 | 6.7 | High | 289 |
| 8 | Bovine | Hide | Acid | 1.55 | 5.4 | Low | 46 |
| 9 | Bovine | Hide | Lime | 4.64 | 5.1 | High | 228 |
| 10 | Bovine | Hide | Lime | 1.83 | 5.0 | Low | 60 |
| 11 | Porcine | Bone | Acid | 2.95 | 7.4 | High | 240 |
| 12 | Porcine | Bone | Acid | 1.90 | 6.8 | Low | 110 |
| 13 | Porcine | Bone | Lime | 2.65 | 4.9 | Low | 86 |
| 14 | Porcine | Skin | Acid | 4.40 | 9.0 | High | 265 |
| 15 | Porcine | Skin | Acid | 2.00 | 8.0 | Low | 75 |

Mixtures, in equal parts, of the industrial batches of bovine or porcine gelatins (2.7 mL solution at 10 mg/mL in phosphate buffer 20 mM pH 7.5) were tyrosylated by adding 0.3 mL of the activated tyrosine. Incubation was done for 2 h at room temperature.

Coupling was stopped by adding 10 mM final hydroxylamine and excess activated tyrosine was eliminated by chromatography on a PD10 column (Amersham Biosciences, 91898 Orsay, France). The coupling efficiency was monitored at 280 nm.

## Polyclonal Antibodies

Bovine and porcine tyrosylated gelatins (9, 15, and 27 μM tyrosine/mg gelatin) were diluted to 1 mg/mL in sodium phosphate buffer (pH 7.2; 0.01 M) containing 0.15 M NaCl (PBS). Rabbits (four for each antigen) were immunized at monthly intervals by multiple intradermal injections of antigen-adjuvant mixture prepared by emulsifying 1 mL antigen sample with 1 mL complete (first injection) or incomplete (booster injections) Freund's adjuvant.[11] Animals were bled seven to nine days after each booster injection and the sera were analysed for antibody activity and specificity by indirect ELISA.



## Indirect ELISA

Polystyrene flat-bottomed microtiter plates (Maxisorp, Nunc, Denmark) were coated overnight at room temperature or 2 h at 37°C with 100 μL of bovine or porcine gelatin in PBS. The optimum coating concentration and pH were determined by checkerboard titration. All subsequent steps were performed at room temperature. Plates were washed five times with PBS containing 0.1% (v/v) Tween 20 (PBSTw). When blocking, the plates were incubated for 30 min with 200 μL of 0.5% HSA in PBS. After five washes with PBSTw, 100 μl of adequately diluted rabbit anti-peptide or anti-tyrosylated gelatins antisera were added to the coated wells. After mixing, the plates were incubated for 1 h and washed five times with PBSTw. Adequately diluted peroxidase-labelled goat anti-rabbit IgG (100 μL) was added and incubated for 1 h in the dark. After five washes with PBSTw, 100 μL of TMB substrate were added, running the color reaction for 30 min before stopping it with 0.36 M $H_2SO_4$ (50 μL/well). Absorbance was measured at 450 nm using a microplate reader (IEMS, Labsystem, Finland). PBSTw with 0.1% (v/v) HSA (PBSTw-HSA) was used as diluent for the solutions. All analyses were performed in duplicate.

## Competitive Indirect ELISA

Plates were coated, blocked, and washed as described for indirect ELISA. PBSTw-HSA was also used as a diluent for the gelatins and antibodies

solutions. While coating and blocking, one volume of each adequately diluted antiserum was incubated in 1 mL test tubes with one volume of serially diluted solutions of gelatins. After 60 min incubation, 100 μL of the antigen-antibody mixture were added to the wells for a 1 h incubation. After five washes with PBSTw, 100 μL of adequately diluted peroxidase labelled goat anti-rabbit antibody were added to the wells and the plates were incubated for 1 h in the dark. All subsequent steps were unchanged. All analyses were performed in duplicate.

## RESULTS

### Reactivity of the Antibodies Raised Against Tyrosylated Bovine Gelatins

The antibody response was monitored by indirect ELISA using the bovine gelatin from Sigma, coated at 50 μg/mL. At the first sampling (one week after the first boost), antibody responses were observed for the gelatin tyrosylated at 15 μM tyrosine per mg gelatin with large differences among rabbits (Fig. 1a). No significative antibody response was obtained with gelatins tyrosylated at 9 or 27 μM.



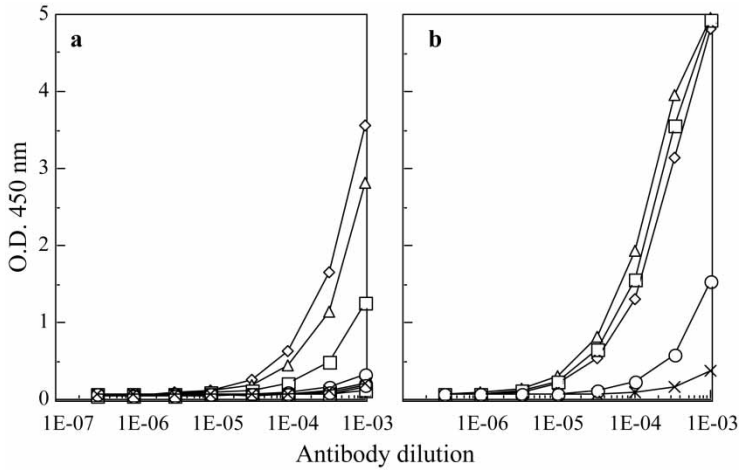


***Figure 1.*** Quantitation by indirect ELISA of the antibody titer of four rabbits immunized against tyrosylated bovine gelatins (15 μM tyrosine per mg gelatin). Sampling was done after the first (a) and second (b) boost. Microtiter plates were coated with the bovine gelatin from Sigma at 50 μg/mL. A peroxidase labelled goat anti-rabbit IgG was used as a tracer antibody. x: normal rabbit serum. Results are means of duplicates.

After the second boost with 15 μM tyrosylated gelatin, high antibody titers were obtained on three of the rabbits (Fig. 1b). The lowest antibody response was around 30-fold lower than that of the highest one.

The antibody specificity of the antibodies after the second boost was tested by competitive indirect ELISA using microtiter plates coated with bovine (Fig. 2a) or porcine gelatin from Sigma (Fig. 2b). As an inhibitor antigen, we tested the 15 batches of industrial bovine and porcine gelatins

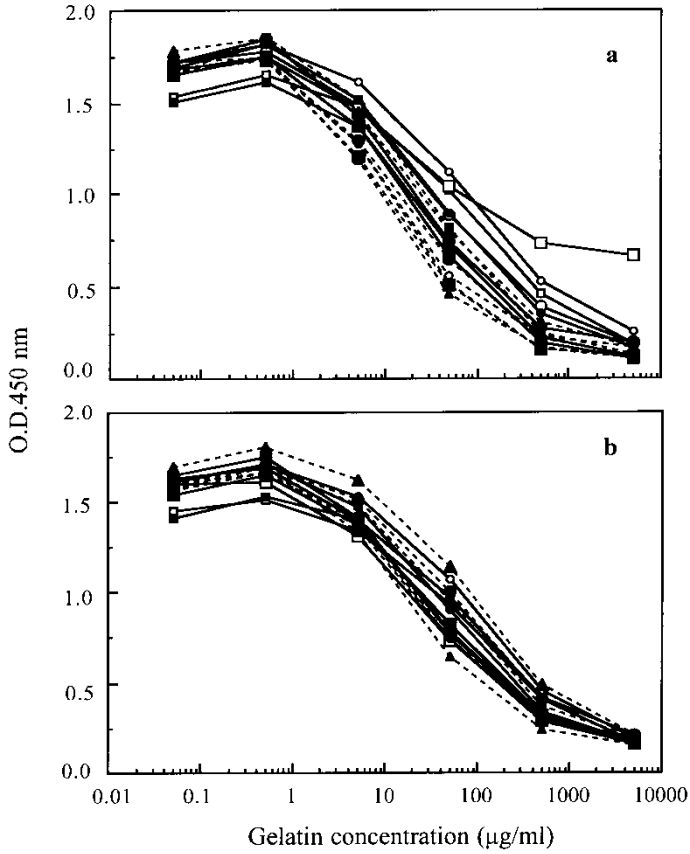


***Figure 2.*** Quantitation by competitive indirect ELISA of bovine and porcine industrial batches of gelatin using rabbit antibodies raised against tyrosylated bovine gelatins (15 μM tyrosine per mg gelatin). Microtiter plates were coated with bovine (a) or porcine (b) gelatin from Sigma at 50 μg/mL. The rabbit anti-bovine tyrosylated gelatins was used as a primary antibody and peroxidase labelled goat anti-rabbit IgG as a tracer antibody. Competitive antigens were bovine (closed symbols) or porcine (open symbols) gelatins obtained by acid (plain lines), lime or sodic (dashed lines) treatment of bones (circles), pig and bovine acid skin/hide (squares) or bovine limed hide (triangles). High Bloom: large symbols; low Bloom: small symbols. See Table 1 for full description of the gelatins. Results are means of duplicates.

covering both hide/skin and bone origin, acid or alkaline processes and high or low Bloom (Table 1). With bovine gelatin coated plate, the inhibitory activity of the porcine gelatins was generally slightly lower than the inhibitory activity of the bovine gelatins. On the porcine gelatin coated plate, similar inhibition curves were observed between the gelatins batches.

After the third booster injection, the major increase in antibody titers was obtained for the rabbit previously giving the weakest antibody response (Fig. 3). However, large differences in antibody specificity were observed between the rabbits antisera when analysed by the competitive indirect ELISA using bovine gelatin coated plates (Fig. 4). Rabbit no. 2 antibodies were more inhibited by the bovine acidic gelatins than by the porcine ones (Fig. 4a). However, these antibodies were highly sensitive to the tissue and the process used to manufacture the gelatin. Bone gelatins were generally more reactive than skin gelatins and acidic gelatins were particularly more reactive than limed gelatins. Thus, the porcine acid bone high Bloom gelatin was $\geq$1,000-fold more reactive than the porcine acid skin high Bloom gelatin. The bovine acid bone high Bloom gelatin was 100-fold more reactive than its limed counterpart and the bovine acid hide high Bloom gelatin reactivity was 1,000-fold higher than that of its limed counterpart. An extreme 4,000-fold difference in reactivity was observed between bovine limed hide high Bloom gelatin and bovine acid bone high Bloom gelatin. Rabbit number 3 did not exhibit such large differences in reactivity, while being slightly less sensitive to the porcine gelatins (Fig. 4b). Rabbits numbers 1 and 4 exhibit intermediate reactivity (not shown).



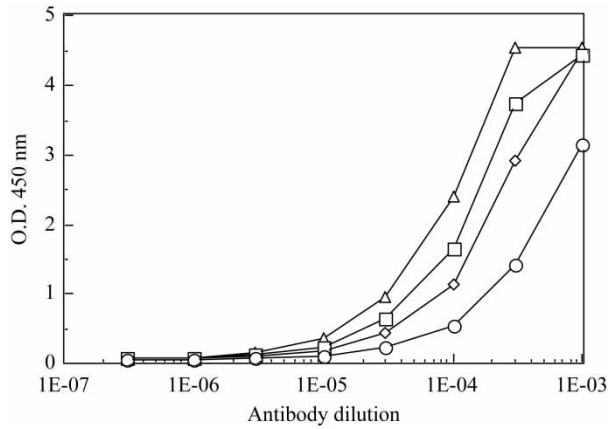


***Figure 3.*** Quantitation by indirect ELISA of the antibody titer of four rabbits immunized against tyrosylated bovine gelatins (15 μM tyrosine per mg gelatin) after the third boost. Microtiter plates were coated with bovine gelatin from Sigma at 50 μg/mL. A peroxidase labelled goat anti-rabbit IgG was used as a tracer antibody. Results are means of duplicates.

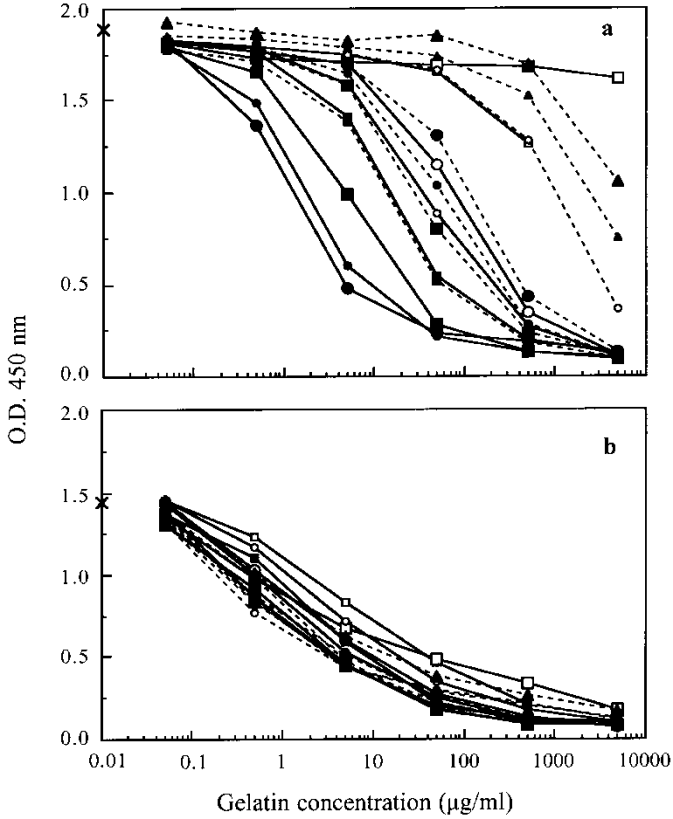


***Figure 4.*** Quantitation by competitive indirect ELISA of bovine and porcine industrial batches of gelatin using rabbit no. 1 (a) or rabbit no. 4 (b) antibodies against tyrosylated bovine gelatins (15 μM tyrosine per mg gelatin). Microtiter plates were coated with bovine gelatin from Sigma at 50 μg/mL. Rabbits anti-bovine tyrosylated gelatins were used as primary antibodies and peroxidase labelled goat anti-rabbit IgG as a tracer antibody. Competitive antigens were bovine (closed symbols) or porcine (open symbols) gelatins obtained by acid (plain lines), lime (dashed lines) or sodic (triangles with plain lines) treatment of bones (circles) or hide/skin (squares and triangles). High Bloom: large symbols; low Bloom: small symbols. See Table 1 for full description of the gelatins. Results are means of duplicates.

Similarly, when tested against porcine gelatin coated microtiter plate (Fig. 5) the rabbits antisera against tyrosylated gelatins exhibited a great variability in titer and specificity. No difference in the inhibitory activity of the 15 batches of bovine and porcine industrial gelatins was observed with rabbit no. 1 (Fig. 5a). In contrast, rabbit no. 2 was very sensitive to the alcaline or acidic process used for the production of gelatins, whatever their species origin (Fig. 5b). Thus, the inhibitory activity of the bovine limed hide low Bloom gelatin was 200-fold lower than its acidic counterpart and the

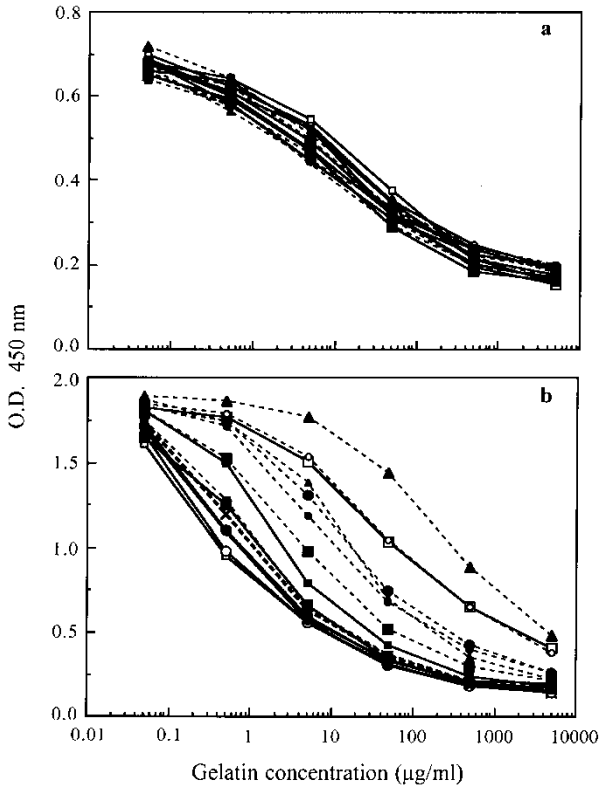


***Figure 5.*** Quantitation by competitive indirect ELISA of bovine and porcine industrial batches of gelatin using rabbit no. 1 (a) or rabbit no. 2 (b) antibodies against tyrosylated bovine gelatins. Microtiter plates were coated with porcine gelatin Sigma at 50 μg/mL. The rabbits anti-tyrosylated bovine gelatins were used as primary antibody and peroxidase labelled goat anti-rabbit IgG as tracer antibody. Competitive antigens were bovine (closed symbols) or porcine (open symbols) gelatins obtained by acid (plain lines), lime (dashed lines) or sodic (triangles with plain lines) treatment of bones (circles) or hide/skin (squares and triangles). High Bloom: large symbols; low Bloom: small symbols. See Table 1 for full description of the gelatins. Results are means of duplicates.

inhibitory activity of the porcine limed bone low Bloom gelatin was 100 fold lower than its acidic counterpart.

## Reactivity of the Antibodies Raised Against Tyrosylated Porcine Gelatins

The antibody response was monitored by indirect ELISA using a coating with porcine or bovine gelatin from Sigma. High antibody titers were observed

essentially against the porcine gelatin (Fig. 6). However, when testing all available batches of industrial gelatins as coating antigens, a process-specificity was observed for rabbits no. 1 and no. 4 (Fig. 7) since their reactivity was generally higher for the acidic gelatins than for their basic counterparts.

Results obtained using the competitive indirect ELISA with Sigma porcine gelatin coated plates confirmed the process and species specificity of these two antisera (Fig. 8). As examples of process specificity, the reactivity of the porcine acid bone low Bloom gelatin was 20,000- (rabbit 1) to 30,000- (rabbit 4) fold higher than that of its limed counterpart; the reactivity of bovine acid bone low and high Bloom gelatins was 1,000-fold higher than that of their limed counterparts and the reactivty of the bovine acid hide was 100- (rabbit 1) to 1000- (rabbit 4) fold higher than that of its sodic counterpart. The species specificity was less marked since the reactivity of the porcine acid gelatins was 10- to 30-fold higher than that of the most reactive bovine gelatins (acid bone low Bloom) (Fig. 8a and 8b). However, the porcine limed bone gelatin was not differentiated from the bovine gelatins.



## DISCUSSION

Collagen is a family of fibrous proteins having a very high tensile strength that are found in connective tissues such as the organic matrice of bones, hides and skins, tendons and cartilage. The structural unit of collagen is tropocollagen, a

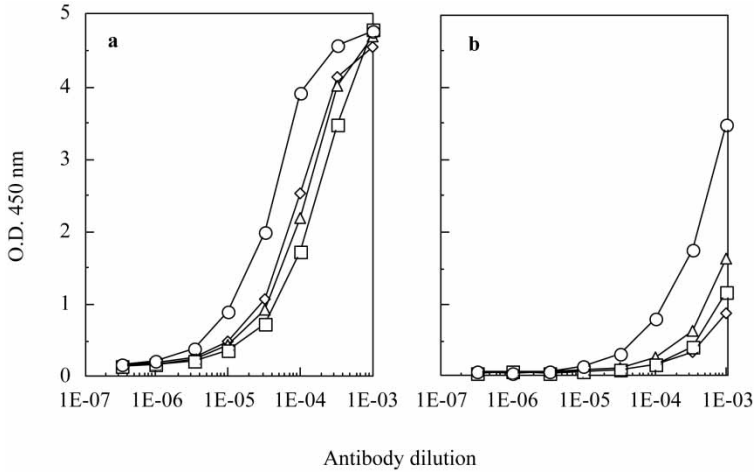


***Figure 6.*** Quantitation by indirect ELISA of the antibody titer of four rabbits immunized against tyrosylated porcine gelatins. Microtiter plates were coated with porcine (a) or bovine (b) gelatin from Sigma at 50 μg/mL. A peroxidase labelled goat anti-rabbit IgG was used as a tracer antibody. Results are means of duplicates.

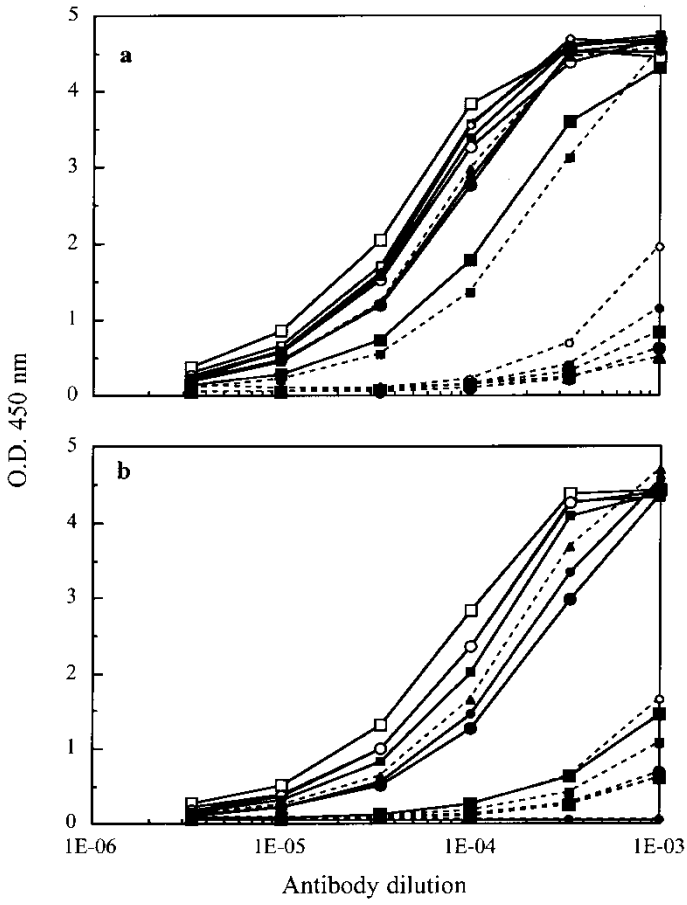


*Figure 7.* Reactivity in indirect ELISA of rabbit no. 1 (a) or rabbit no. 4 (b) anti-tyrosylated porcine gelatins tested against industrial batches of bovine and porcine gelatins. Microtiter plates were coated with bovine (closed symbols) or porcine (open symbols) gelatins obtained by acid (plain lines), lime (dashed lines) or sodic (triangles with plain lines) treatment of bones (circles) or hide/skin (squares and triangles). High Bloom: large symbols; low Bloom: small symbols. See Table 1 for full description of the gelatins. A peroxydase labelled goat anti-rabbit IgG was used as a tracer antibody. Results are means of duplicates.

protein formed of three helical units wrapped around one another with a right handed twist. Each of theses helices contains about 1,050 aminoacids with a highly distinctive sequences: nearly every third residue is glycine (21–35%). Other important aminoacids are alanine (9–11%), proline (12%), the unusual hydroxyproline (9–12%), and a few percent of hydroxylysine.[12]

Gelatin is a randomly coiled protein obtained by denaturation of collagen derived from sources rich in type I collagen, such as skin and bones. Two main

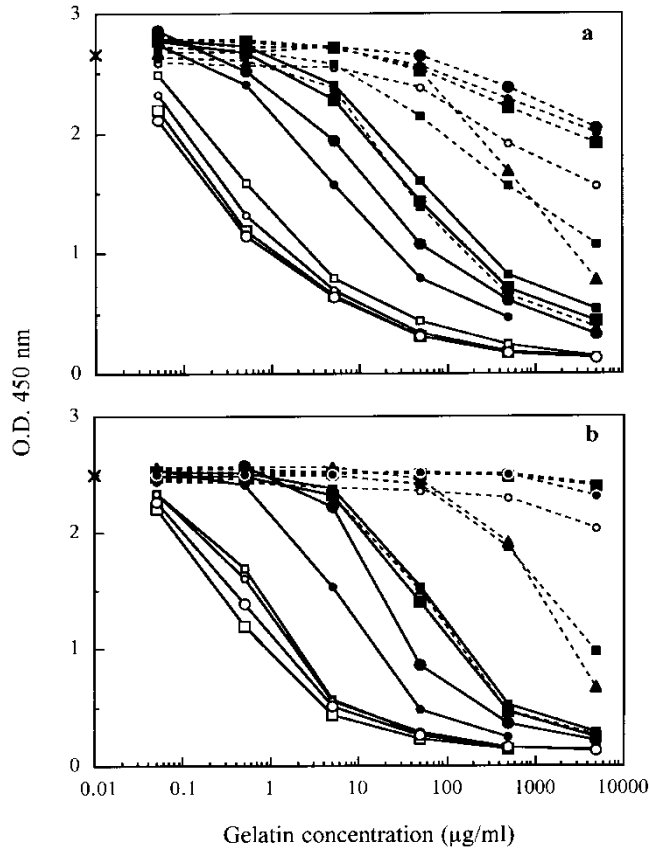


***Figure 8.*** Quantitation by competitive indirect ELISA of bovine and porcine industrial batches of gelatin using rabbit no. 1 (a) or rabbit no. 4 (b) antibodies against tyrosylated porcine gelatins. Microtiter plates were coated with porcine gelatin from Sigma at 50 µg/mL. The rabbits anti-tyrosylated bovine gelatins were used as primary antibodies and peroxidase labelled goat anti-rabbit IgG as a tracer antibody. Competitive antigens were bovine (closed symbols) or porcine (open symbols) gelatins obtained by acid (plain lines), lime (dashed lines) or sodic (triangles with plain lines) treatment of bones (circles) or hide/skin (squares and triangles). High Bloom: large symbols; low Bloom: small symbols. See Table 1 for full description of the gelatins. X: no competitive antigen. Results are means of duplicates.

types of gelatins are produced: type A, with isoionic point of 7 to 9, is derived from collagen with exclusively acid pretreatment; type B, with isoionic point of 4.8 to 5.2, is the result of an alkaline pretreatment of the collagen. Gelatin is sold with a wide range of special properties, like gel strength, to suit particular applications.

The diversity in collagen origin (bone/hide/skin) and processes applied (acid or lime treatment) explains the difficulties encountered for the

determination of the species origin of gelatins using physico-chemical methods[2,3] and the need of more specific approaches, such as immunochemical techniques. However, a major difficulty in using this approach is the well known weak immunogenicity of collagen.[13] Sucessfull immunization usually followed the administration of soluble collagen, but antibody titers were often low, even after long periods of immunization. The weak antigenicity of collagen has been related to low aromatic and sulphur content, high imino acid content, ability to resist the degradative power of proteolytic enzymes, and failure of recognition mechanisms in closely related species.[14] However, antigenic determinants have been described on the collagen molecule.[15,16]

Gelatin, as a denaturation product of collagen is a such poor antigen that, for many years, its immunogenicity escaped notice. Sela and Arnon[10,17,18] have demonstrated that the enrichment with tyrosine converted gelatin into a relatively powerful antigen. Furthermore, they have found that the serological specificity of the gelatin derivatives changes strongly as a function of their tyrosine contents. The specificity of compounds rich in tyrosine resides almost exclusively in the peptide chains attached, whereas it resides essentially in the gelatin moiety in the derivative containing only 2% of tyrosine residues.[10,17] Kirrane and Robertson[14] obtained comparable results with tyrosylated rat collagen.



As the extent of gelatin tyrosylation is critical for the antibody titer and specificity, we immunized rabbits with bovine or porcine gelatins tyrosylated at different ratios. One of them (15 μM tyrosine/mg gelatin) was successful since antibodies reacting with the native gelatins were detected as soon as one week after the first boost, and high titers were obtained at the second boost. These results confirm the possibility to raise high titer antibodies against bovine or porcine gelatins by using controled tyrosinylation, as described by Arnon and Sela.[10]

However, we observed, among rabbits and during the course of immunization, large differences in the specificity of antisera. Some of the antisera were not species or process sensitive and could be used to control the presence of gelatin in food, whatever its animal origin. Unexpectedly, other antisera were highly sensitive to the acid or alkaline processes used for gelatin production, while they were obtained by immunization with mixtures in equal parts of the industrial batches of bovine or porcine gelatins obtained by acid or lime treatment. The process effect was particularly marked for the porcine gelatins, since two of the four rabbits immunized against porcine tyrosylated gelatin detected the acid porcine gelatins with a 20,000–30,000 higher sensitivity than the limed one.

This sensitivity to the process was detected by indirect ELISA, as well as by competitive indirect ELISA, and thus cannot be related to possible conformational changes of the gelatins upon coating in the indirect ELISA. However, it could be related to conformational changes occuring as a consequence of the denaturating effect of the process, since limed gelatins are less

immunoreactive than acid gelatins. During the lime treatment of collagen, the helical structure of the chains is generally preserved, the crosslinks are broken, and the glutamine and asparagine residues are progressively desaminated. Thus, the loss of immunoreactivity of the lime processed gelatins could be consecutive to these amino acid modifications and/or to changes in the three-dimensional structure of the molecule after breaking of the interchains cross-links. Moreover, it can also be postulated that the tyrosylation of the limed gelatins has not been as effective as the tyrosylation of the acid gelatins, due to the chemical modification of $-NH_2$ bearing amino-acids in limed gelatins. Whatever the explanation, such antisera could be used as very sensitive probes to monitor the denaturation of the collagen molecule during its transformation to gelatin.

The species sensitivity of antisera raised against the bovine and porcine tyrosylated gelatins was less marked than their process sensitivity. The antisera raised against the bovine tyrosylated gelatins were generally more sensitive to the bovine gelatins than to the porcine ones. However, these antisera could not be used for a sensitive differentiation between the two species, due to the superimposed process sensitivity that made the porcine gelatins react like the limed bovine gelatins. In contrast, antisera raised against the porcine tyrosylated gelatins were more species specific since their reactivity to the porcine gelatins was 10- to 30-fold higher than that of the most reactive bovine gelatins. Only the porcine limed gelatin bone was not differentiated from the bovine gelatins. Such antisera could, thus, be used to control the presence of porcine acid gelatins in bovine gelatins. Detection of porcine acid gelatins in food could be obtained by comparing the results obtained for total gelatin using the non-species and process sensitive antisera with that obtained using the porcine gelatins sensitive antisera.

The relative low species sensitivity of the obtained antisera can be easily explained by the exceptionally high homology of published sequences of collagen between different species. As an example, we found a 97% homology between the alpha 1(I) chain of the bovine and human collagen. Thus, in order to obtain more species specific antisera, rabbits should be immunized with synthetic peptides carefully designed to mimick restricted species specific areas of the collagen chains. Works in progress in our laboratory indicate that this approach is effective in obtaining bovine gelatin specific antisera with low process sensitivity.



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