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SPECIFIC SEROLOGICAL IDENTIFICATION OF OSTRICH MEAT AND MEAT PRODUCTS

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SUMMARY

Using aqueous urea solution extracts of heated ostrich muscle as antigen for the production of precipitating rabbit anti-ostrich sera, it was possible to specifically identify raw, heated (70-95°C) and air dried-salted ostrich meat by means of gel immunodiffusion tests. The sera did not react with chicken, turkey or horse meat or with beef in any form.

The soluble proteins extracted from ostrich meat heated to temperatures of 70°C for 30 minutes appear to constitute at least two closely related antigenic determinants of which only one is thermostable at temperatures above 70°C.

INTRODUCTION

Food legislation requires that "meat other than that of bovines, sheep, pigs and goats shall bear a label indicating its nature"; this also applies to any preparation or mixture of meat, processed meat and manufactured meat products[2].

The ostrich *Struthio camelus* Linn. is indigenous to Southern Africa and it's domestication has led to commercialisation and the production of meat for human consumption. Apart from conventional cooking of fresh meat a considerable proportion is converted into biltong by salting and air drying the raw or partially heated meat.

Positive identification of several large consignments of suspect ostrich biltong became necessary for forensic purposes. Efforts to do so by the use of aqueous extracts of the biltong against commercial anti-avian sera were unsuccessful (Brig. L. Neethling, S.A. Police Labs., personal communication.) It was suspected that the meat had been heated before salting and drying.

Serological identification of raw food animal meats is well known and frequently employed, and reports have also appeared concerning the identification of biltong[3 6 7]. Serum was traditionally used as antigen for antiserum production, with aqueous extracts of homologous meat providing sufficient serum proteins for a visible homologous precipitin reaction. For heat processed meat this method was less than satisfactory, i.a. because of the heat lability and the low concentration of serum proteins obtained by simple aqueous extraction of muscle.

Sinell and Mentz reported that high concentrations of sarcoplasmic proteins, as determined by the Biuret method, could be obtained by the use of 6 M urea solutions to extract both raw and heat processed meats[5]. The urea was found not to alter the relevant characteristics of native material nor to affect species specificity. Such urea extracts were eminently suitable for use as antigens in the production of antisera which reacted well with urea extracts of both native and heated meat (up to 120°C).There was, however, a clear difference between the electrical mobility of the heated and native homologous material, the former being a highly thermostable protein[5]. These findings have led to important modifications of earlier serological methods for species identification of meat.

With the exception of a single reference to the failure of extracts of ostrich biltong to react positively with precipitating sera produced by immunisation of rabbits with bovine serum[7], no other reports on ostrich meat identification have appeared.

Positive identification of raw, heated and air dried salted ostrich meat (biltong), and differentiation between ostrich meat and that of bovines, equines, chickens and turkeys is the subject of this report.

MATERIAL & METHODS

1 Extracts of muscular tissue were prepared according to the method described by Sinell and Mentz[5]; this included mincing, homogenisation in dry ice, extraction in 6 M urea solution, lyophilisation and reconstitution for use as antigen for antiserum production and as antigen for gel diffusion tests. Extracts were similarly made from minced meats heated for 30 minutes to 70°, 80°, 90°and 95°C, from biltong prepared from raw and heated beef, horse meat and ostrich meat, and from the biltong of unknown derivation. The protein content of such extracts was determined by means of the Biuret reaction method and found to fall within the 400-800 ug N/ml recommended by H.J. Sinell (personal communication.)

2 Antisera were produced by injection of rabbits with urea extracts of various meats, with and without Freund's complete adjuvant, using a system advocated by Sinell & Mentz[5]. Intraperitoneal booster injections of 3 ml of antigen were given subsequently on three occasions at weekly intervals. The resulting antisera gave clear and well defined precipitin reactions against autologous and homologous meat extracts in gel diffusion plates.

3 Ouchterlony double diffusion agar gel plates of 1 mm thickness were prepared immediately before use from 1% solutions of Special Noble agar (Difco) in phosphate buffer. Wells of 3 mm diameter were set into the gel by means of a standard punch at equal distances around a central well. After filling the wells with antigen or antiserum the plates were incubated at 27°C for 24 h before being processed and read.

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4 Electrophoresis plates were prepared on standard microscope objective glass slides, each having a 6,5 mm central longitudinal trough situated equidistant between two 1 mm diameter antigen wells. After filling the wells the plates were placed in an electric field of 250v/50 mA for 1,5 h and therafter 0,04 ml of antiserum was placed in the trough for final diffusion at 27°C for 20 h as described by Sinell and Kluge - Wilm[4].

RESULTS AND DISCUSSION

1 On the Ouchterlony double diffusion agar plate a thick apparently single and a thinner precipitin line developed when testing rabbit anti-ostrich meat serum against the homologous meat extract and against an extract of biltong made from ostrich meat. No lines became visible when testing this antiserum against heterologous meats, i.e. that of equines, bovines, turkey and chicken.

2 On immunoelectro-osmophoresis two distinctly separate precipitin lines could be demonstrated when testing rabbit anti-heated ostrich meat serum (RAHOS) against the homologous meat extract; no lines developed against heterologous meats (See Fig. 1). According to Clausen[1] the double-humped arcs indicate the presence of an antigen with two main electro-phoretic mobilities but possessing identical or partially identical antigenic properties.

Fig. 1
Agar gel diffusion, by electrophoresis, of rabbit anti-heated ostrich meat serum (RAHOS) against heated ostrich meat extract (HOME) and heated beef extract (HBE).

3 Testing RAHOS prepared from meat heated to 70°C for 30 minutes against urea extracts of raw ostrich meat (ROME) and ostrich meat heated to 70°, 80°, 90°and 95°C as well as against a urea extract of the unknown biltong, clear precipitin lines developed in all instances (See Fig. 2)

Repetition of this procedure using RABS failed to elicit any visible lines.

From this it was firstly concluded that the unknown biltong was in fact derived from ostrich meat. From the similarity of the lines opposite the unknown biltong well and those opposite the well containing an extract of ostrich meat heated to 70°C it is also concluded that the biltong was made from meat heated to a similar temperature. From Fig. 2 it can be seen that lines opposite ostrich meat heated to a higher temperature were single and less distinct. The spur formation at the point of contact between the lines of precipation opposite the wells containing extracts of meat heated to 70°C and those heated to temperatures above 70°probably results from reaction of the RAHOS with a multiple antigen or an antigen possessing different determinant groups[1]. It would therefore seem that the antigen produced by extraction of water soluble proteins from meat heated to temperatures above 70°C is a single relatively heat stable protein; conversely, extracts of meat which is raw or heated to temperatures below 70°C appear to contain at least three and two antigens respectively, one of which is labile to heat above 70°C.

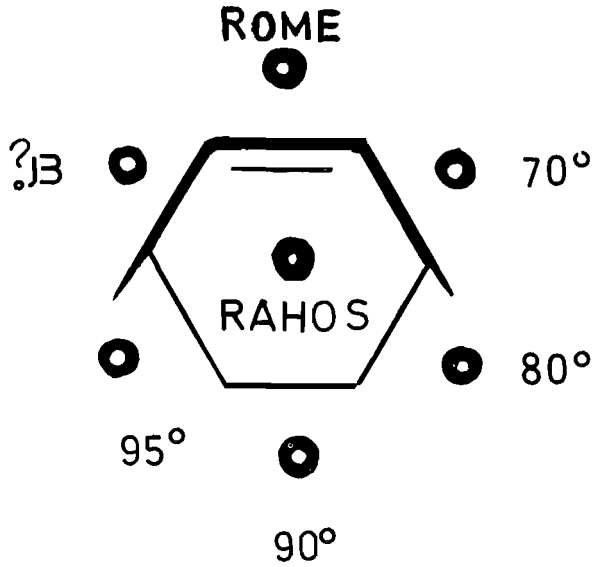

Fig. 2
Representation of double agar gel diffusion test of urea extracts of unknown biltong (?B) and or raw (ROME) and heated (70° - 95°C) ostrich meat against rabbit anti-heated ostrich meat serum (RAHOS)

In contradistinction to Sinell and Mentz[5] it was found that clearly defined precipitin lines were visible in the homologous systems when using the Ouchterlony double diffusion gel method. The duplicity of the antigen determinants resulting from urea extraction of meat on which they report was however confirmed in our studies as was the thermostability of one of the antigen components.

In the double diffusion tests in which RAOS was set up against extracts of raw and heated chicken and turkey meat, no visible precipitin lines could be detected by use of the technique employed. From this is it is concluded that our anti-ostrich serum did not produce cross-reacting precipitin lines with chicken and turkey meat extracts and that it can be employed for distinction between these two avian species and ostrich meat.

In a double diffusion test using rabbit antibeef serum (RABS) centrally and extracts of known beef biltong and the unknown biltong peripherally, a clear precipitin line developed in the homologous system. No precipitin lines were visible opposite the unknown biltong, and this further indicates that the unknown biltong was not derived from beef.

CONCLUSIONS

From the above it is concluded that specific precipitating rabbit antisera can be produced against raw and heated ostrich meat which will positively identify such ostrich meat and also distinguish it from equine, bovine, chicken and turkey meat, whether raw or heated up to 95°C.

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BOOK REVIEW BOEKRESENSIE

THE VETERINARY ANNUAL

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Wright-Scientechnica, Bristol 1975.

pp xviii +483. Figs 120 (4 colour). Tabs 58.

The enlarged fifteenth edition of this well known work is contributed to by no less than 87 distinguished authors and covers most fields of modern veterinary medicine. The clinician is well catered for with succint papers on numerous aspects of surgery, medicine, diagnostics and therapy of both farm and small animals. A review of fish diseases of 19 pages is beautifully illustrated with 16 photographs.

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BOOK REVIEW BOEKRESENSIE

THE USE OF MERCURY AND ALTERNATIVE COMPOUNDS AS SEED DRESSINGS

WORLD HEALTH ORGANIZATION TECHNICAL REPORT SERIES NO. 555, GENEVA, 1974.

pp. 29. Tabs. 6 Publ. price Sw. f. 5.-

Cereal seed must be treated with fungicidal dressing to prevent seedborne infection and to protect germinating seeds from soilborne pathogens. The organomercury compounds, being cheap and having a wide antifungal spectrum, are particularly suitable for this purpose. Such seed is, however, very dangerous if, for various reasons, it should be used as food or feed.

An interesting, brief review of the problem is given in this booklet and the relative dangers of the alkyl-, alkoxyalkyl and arylmercury compounds as well as hexachlorobenzene are discussed. The exceptional danger of alkylmercury compounds is pertinently emphasized.

Recommendations are made on the safe use of these products until suitably effective and cheap replacements have been developed.

T.W.N.

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