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34 (1) : 97-104, 1987

THE EFFECTS OF CURING AND COOKING ON THE DIFFERENTIATION OF SPECIES ORIGIN OF MEAT PRODUCTS BY ISOELECTRIC FOCUSING

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İzoelektrik fokuslama yöntemiyle et ürünlerinin hayvan türlerine göre ayırımında kürleme ve pişirmenin etkisi.

Özet: *Bu araştırma etlerin ürünlerine işlenmeden ve işlendikten sonra türlerine göre izolelektrik fokuslama yöntemiyle ayırımlarında kürleme ve ısı işlemlerinin olası etkilerini saptamak amacıyla yürütülmüştür. Bu amaçla sığır, domuz ve koyun türlerine ait etlerin her birisinden sırasıyla; saf çiğ; saf pişirilmiş; kürlenmiş; ve kürlenmiş–pişirilmiş olmak üzere numuneler hazırlanmıştır. Sonra guanidine hydrochloride ile elde edilen numune ekstrakları adenylate kinase enzimini tesbit etmek için agarose gelde izoelektrik fokuslama yöntemine uygulanmışlardır.*

İzoelektrik fokuslama sonucunda her numune kolaylıkla görülebilecek koyuluk ve büyüklükte adanylate kinase lekesi vermiştir. Gerek aynı türe, gerekse farklı türlere ait numunelerden elde edilen adenylate kinase enzimi leke kalıpları arasında çok az bir fark saptanmıştır. Yalnız koyun numunelerinden elde edilen adenylate kinase enzimlerinin diğer örneklerden elde edilenlerden biraz yüksek izoelektrik noktaya sahip oldukları gözlemlenmiştir. Elde edilen bulgulara göre, bu deneysel koşullarda kürleme ve ısı işlemleri, ürünlere işlenen bu etlerin türlerine göre ayırtedilmelerine önemli derecede etkilememiştir.

Summary: *The purpose of this study was to investigatie the possible effects of curing and cooking on the differentiation of species origine of beef, pork and sheep meats and meat products of these species meats by isoelectricfocusing technique. For this purpose, the samples were*

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prepared from beef, pork and sheep meats as pure raw; pure coked; cured; and cooked and cured. Each sample was extracted with quanidine hydrocloride and the sample extracts were subjected to isoelectricfocusing procedure to stain adenylate kinase in agarose gel.

Each sample extract subjected to isoelectricfocusing yielded sufficiently intense and large enough adenylate kinase band. Very little changes have been observed within and among the isoelectricfocusing band patterns of adenylate kinase of species meats. Adenylate kinase bands obtained from sheep samples showed slightly higher isoelectric points than the other samples. Based on these results, under the conditions of this experiment, curing and cooking did not effect significantly the differentiations of these species meats.

Introduction

Fraudulent substition of low quality meats and vegetable proteins for meat products produced with high quality meats is an ongoing importent problem in the meat industry in many countries (1, 6, 8, 9, 10, 11, 16, 21). Because, numerous methods used for monitoring adulteration problems, particularly identification of undeclared meats in cooked meat products are to some extent inadequate (1, 7, 13, 14, 16, 18).

More recently a great deal of interest and effort over the adulteration of meat products with cheaper species meat has improved some methods particularly isoelectricfocusing (IEF) and enzyme-linked immunosorbent assay (ELISA) for species identification of meats (12, 14, 18). The results reported (8, 11, 18, 19, 21), that staining of IEF and ELISA techniques have porential to differentiate closely related species meat in unheated and heated meat products as well as in raw meats.

ELISA capaple of differentiation of unprocessed beef, sheep, horse, kangaroo, pig, cemal, buffalo and goat meat to less than 1 % level of detection (17, 18, 19, 20). This method has also been shown to be applicable even to mildly heated meat products (4, 15, 16).

Although, ELISA is very economical and permits for identification of species origing of meats in raw meats, unheated and midly heated meat products, species-specific antisera are not easily available

for all species of interest. In addition to his, application of this method to cooked products has been only partially succesful. For this reason, increased attention has been directed towards improving the electrophoretic methods, particularly enzyme staining of IEF in agarose gel (11, 13, 14).

Enzyme staining of IEF in agarose gel gives better protein resulution than IEF in polyacrylamide (13, 14). King (11) and King and shaw (14) were able to determine species origing of cooked meats and detection of species flesh in smallgoods products by staining heat stable enzymes, such as adenylate kinase and creatine kinase after submitting of extracts of meat samples to IEF. But this technique was found unsuitable for detection of more than one species of meat in the mixture of meat products (11).

Consequently, these two techniques are still suffering from several drawbacks due to heating of meat products and adding most of them different types of additives such as salts, species and extenders. These factors cause denaturation, precipitation and / or altering of antigenic properties and isoelectric points of meat proteins (11, 12).

The objective of the present study was to investigate the influence of curing agents and cooking on the differention of species of meats and the meat products by the enzyme staining of IEF technique.

Material and Methods

Preparation of Samples: Beef, pork and sheep meats were obtained from the Muscle Biology Laboratory, University of Wisconsin, Madison. The sample were prepared experimentally from each species of meats in 0.5 kg amounts as shown; 1) pure raw meats; 2) pure-cooked meats; 3) cured meats; and 4) cooked and cured meats. The samples were cured by adding 2.5 gm fast cure mixture W-N series with color (6.22 % sodium nitrite, dextrose, certified food color and less than 1.0 % glycerine to prevent caking). 5.5 gm summer sausage seasoning (coriander, white peper, black peper, nutmeg, 4.58 % mustard seed and allspice) (.E.W. Witt and Company, 1106 S. Bridge Street, Yorville, IL), and 28 gm sodium chloride per kg meat. The meat preparations were stuffed into summer sausage casing and vacuum packed. The samples were then cooked in a water bath at 85 $\pm$ 2°C for 30 minutes. All samples were stored at -20°C until use.

From each sample 10 gm was ground to a fine powder under liquid nitrogen, freeze-dried and stored at -20°C prior to extraction. A portion (50 mg) of the freeze-dried powder sample was extracted with 10 ml solution containing 6 M guanidine hydrochloride, 20 mM tris-(hydroxymethyl) aminomethane and 1 mM dithiotreitol at pH 8.0, by stirring 1 hour at room temperature and then sentrifuged at 50.000 x g for 0.5 hr. at 15°C. After centrigation, supernatent of each sample was dialized against two changes of 1 % Triton x-100, 0.1 % 2-mercaptoethanol over period of 18 hrs as describe by Kint (11).

Isoelectricfocusing: Carrier ampholytes (pharmalyte pH 5-8 and 8-10.5) were obtained from Pharmacia (pharmacia AB, Uppsala, Sweden). IsoGel agarose and GelBon film were obtained from FMC (FMC Co., Rockland, ME, U.S.A.). Agarose gels (125 x 100 x 0.75 mm) containing 1 % agarose, 1 % Triton X-100 and 3.3 % carrier ampholytes were formed on Gelbond film by a procedures described in FMC instruction sheet (3). The GelBond supported IEF gel was placed on the cooling platform of horizantal electrophoresis cell (Model 1415, Bio-Rad Laboratories, Richmond, CA. U.S.A). The electrode strips soaked in 1.0 M O-phosphoric acid (anolyte) and 1.0 M sodium hydroxide (catholyte) were placed on the gel surface. Electrofocusing electrodes were put on to the electrode strips. 2 µl of each sample extract was applied on the gel surface by means of a micropipet approximately 2 cm from anode. Water was circulated through the cooling unit at 10°C while power was applied by electrophoresis power supply (Model 494, ISCO 1400, Lincoln Nebraska, U.S.A). Power of 1 W was applied to 30 min initially and then the power was increased to 10 W for a further 1 hr.

Staining procedure: Gel was stained for adenylate kinase (myokinase) activity by agar overlay technique according to procedure described by Harris and Hopkinson (5). The substrate solution given below was prepared as describe by King (11).

Fifty µl of hexokinase-glucose-6-phosphate denydrogenase,1 ml nitrobluetetrazolium (1 % in water), 1 ml phenazine methosulphata (0.2 % in water) and 40 ml agar (2 % in water, dissolved by boiling and then cooled to 60'C) were added respectivelly to 20 ml substrate solution containing 5 mM adennosine-5'-diphosphate, 0.1 M glucose, 0.2 mM β-nicotinamide-adenine dinucleotide phosphate, 5 mM magnesium sulphate and 0.2 M tris-(hydroxyamino)

methane at pH 7.8. This substrate solution was poured as an overlay on the agarose gel after isoelectricfocusing. The gel was then placed as soon as possible in a dark incubator at 45°C. Bands appeared approxymately within 15 minutes. When bands reached in the desired staining intensity, the formazon reaction was stopped by immersing the gel in solution containing 1.5 % acetic acid and 1 % methanol in the dark. This solution was changed several times over a period of 2 days. The gel was then dried on the gelBond film at 60°C in an oven with fan-driven air circulation.

Results

Adenylate kinase band patterns obtained from the samples which were prepared from three species of meats (beef, pork an sheep) as pure raw; pure cooked; cured; and cured-cooked are illustrated in Fig. 1. Each sample extract subjected to enzyme staining of IEF procedure yielded sufficiently intense and clearly appearred adenylate kinase band. As it is seen from fig. 1. addition of curing agents and application of heat to the samples separately and together did not effect significantly the appearence of adenylate kinase bands and also not altered isoelectric point of this enzyme. Very little changes have been absorved within and among the isoelectric focusing patterns of adenylate kinase of beef, park and sheep samples. Adenylate kinase bands obtained from sheep samples showed a little more migration or higher isoelectric points than pork and beef samples. Beef samples revealed lesser intense adenylate kinase bands than the other samples.

Discussion

The present study was counducted to investigate the possible effects of curing and cooking on the differention of species origin of beef, pork and sheep meats and meat products prepared from these species meats.

All samples extracted with guanidine hydrochloride and then subjected to enzyme staining of IEF in agarose gel yielded clearly defined adenylate kinase band patterns (fig 1.). Adding the curing agents (NaCl and $NaNO_3$) and applying the heat (at 85°C for 30 min) to the samples separately and together did not altered the formation of adenylate kinase bands of samples in agarose gel. These findings

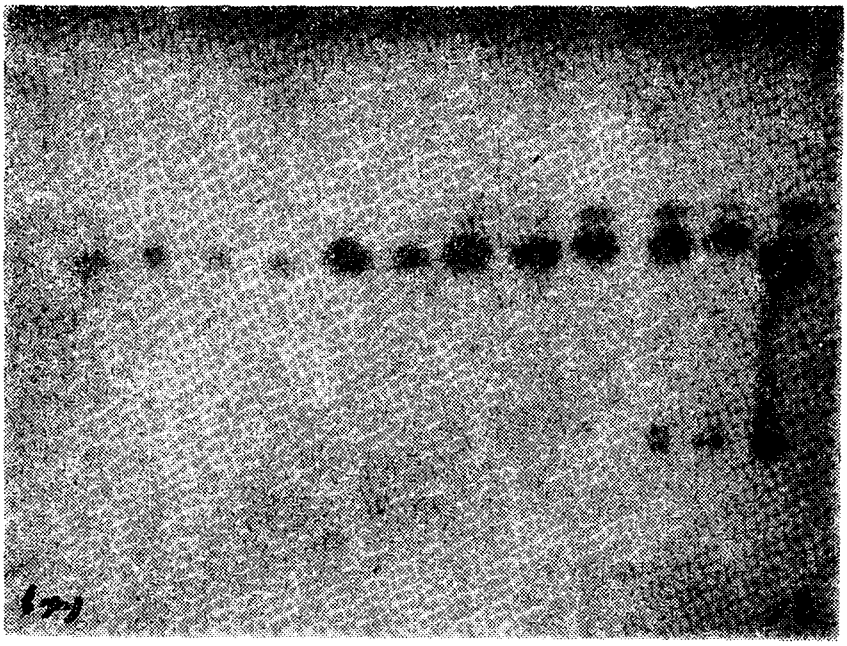

Figure 1. Isoelectricfocusing gel, pH range 5–10.5; stained for the enzyme eadenylate kinase: a) pure raw beef; b) pure cooked beef; c) cured beef; d) cooked and cured beef; e) pure raw pork; f) pure cooked pork; g) cured pork; h) cooked and cured pork; i) pure raw sheep; j) pure cooked sheep; k) cured sheep; l) cooked and cured sheep meat.

in this study are in agreement with those published before (11, 12, 13). They concluded that the differentions of species origin of raw meats and meat products cured and heated up to 100°C could be possible by staining for particular enzymes such as adenylate kinase, creatine kinase and phosphogluconate dhydrogenase.

All combinations of beef, pork and sheep meat samples yielded slightly different isoelectricfocusing adenylate kinase band patterns. On the other hand, the isoelectric points of adenylate. kinase obtained from each sample were very close to each other. For that reason, to distinguish these species meats and meat products prepared from them were not possible clearly. As a matter of fact, a study done before reported that beef, sheep and pork have similar adenylate kinase band patterns (14). Obtaining the similar adenylate kinase band patterns migth be arose by the preparing of the samples from phylogenitically related species meats.

Consequently, heating at 85°C for 30 min. and adding the salts (NaCl and $NaNO_3$) with other additives did not potentially alter the isoelectric points of adenylate kinase extracted from feef, pork and sheep meats, However, additional researches sould be carried out by staining for various enzymes after submitting quanidine hydrochloride extraction of more species meats products samples to isoelectrict focusing in agarose gel.

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