Quantitative analysis of raw meat mixtures of pork and beef by isoelectric focusing in agarose gel.

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SUMMARY

Isoelectric focusing of raw meat extracts on thin layers of agarose followed by densitometry of two specific pork and beef protein bands allow a routine estimation of the beef-pork composition. Using the relative difference in absorbance of two pairs of pork and beef protein bands the composition of 50 different beef-pork mixtures was accurately determined. The method can be extended to other raw meat (e.g. chicken-pork or horse-beef) mixtures.

INTRODUCTION

Isoelectric focusing of raw skeletal extracts in polyacrylamide (PAGIF) or in agarose (AGIF) gels are excellent methods for identification of fish (1, 4, 9, 10) or animal species (2, 4, 8). Densitometric evaluation of species specific protein bands in the pherogram would allow quantitative analysis of binary meat mixtures (5). Kaiser et al. (5) used the ratio of the peak areas of a specific protein band of pork and beef after AGIF in pH 3.5 - 9.5 gels.

We studied the absorbance of some specific protein bands of pork and beef in extracts from different muscles and animals after AGIF in the pH-range 5.0 - 8.0. The absorbance

of the protein bands studied varied widely depending e.g. on the individual muscle or carcass. Thus estimation, based on the ratio of peak areas, may be very inaccurate. It is shown that relative difference in absorbance between a specific protein band of pork and beef is directly related to the relative dilution of beef in pork and may be used as a reliable parameter for the percentage composition of beef-pork mixtures.

MATERIALS AND METHODS

Agarose EF and carrier ampholyte pH 5.0 - 8.0 were purchased from LKB Produkter, AB (Bromma, Sweden). Coomassie brilliant blue R 250 was obtained from Serva (Heidelberg, GFR). Other reagents were from E. Merck (Darmstadt, GFR).

Muscles (breast, thigh, neck, M. diaphragma, M.L. Dorsi) were sampled in the slaughterhouse from different carcasses of beef (10) and pork (7) and frozen at -20 °C until analyzed. Meat mixtures were prepared by homogenizing a suitable amount of a separate muscle of beef and pork so that each mixture contained muscles of different origin. Meat extracts were prepared by homogenization of raw meat in an equal weight of ice-cold water by Ultra-Turrax. After centrifugation (20 min., 10 000 g at 2 °C) the supernatant was decanted over glass-wool. Aliquots of the extracts (dilution 1/2) were diluted two- (1/4), four- (1/8) and eight-fold (1/16) and stored at -20 °C until analysis.

AGIF-electrophoresis of the aqueous extracts was carried out according to LKB-instructions (7) at 10 °C. Gel slabs (125 x 240 x 0.5 mm) were casted on gelbound film using carrier ampholytes (pH 5.0 - 8.0) for agarose IEF. 20 µl (or 10 µl) of the sample extract was pipetted on filter paper (1.5 x 1 cm resp. 0.75 x 1 cm) and the sample applied at 15 mm from the cathode. Power supply was set at 7 W during 15 min. after which the strips were removed. Focusing was performed during 60 min. at 7 W followed by 10 min. at 10 W. Fixing, washing, staining and destaining of the gels was similar to LKB-instructions.

Different staining procedures were compared in which the time of fixation (5 - 40 min.), washing (5 - 20 min.) and staining (10 - 60 min.) was varied using either CBB R 250 at 0.25 % or 0.5 %. On one gel three series of beef-pork extracts (beef-pork mixtures of 100:0 ; 80:20 ; 60:40 ; 40:60 ; 20:80 and 0:100) at dilution 1/4 were applied on the plate. After AGIF, the plate was cut in three parts : two series of extracts were stained by two different staining procedures ; the LKB-staining was taken as reference. The optimal staining procedure was : 20 min. fixation in 10 % tricloracetic acid and 1 % sulfosalicylic acid, 5 min. washing in 95 % ethanol, 60 min. staining in 0.25 % CBB R 250 in destaining solution and destaining during 1.5 - 3 h. in 10 % acetic acid in 33 % ethanol. After drying the gels were scanned in transmission using a chromatogram KM 3 Zeiss-scanner at 546 nm with a slit at 0.05 mm. Peak areas were calculated by triangulation. The relative mobilities of the protein bands were calculated as the ratio of the distance of the band from the cathode to the distance between cathode and anode.

RESULTS AND DISCUSSION

1. Staining characteristics of the species specific protein bands :

The staining characteristics of the various protein bands depend upon experimental staining conditions e.g. time of fixing, staining with CBB R 250 dye and destaining. Since it was observed that different pork protein bands stain and destain faster than the beef bands, the staining procedure was critically examined for optimal coloration, reproducibility, linearity of absorbance over the concentration range and background coloration. Three typical pork (Fig. 1 : P_1, P_2, P_3) and beef bands (Fig. 1 : B_1, B_2, B_3) from pork-beef extracts were scanned and evaluated by densitometry. Since in AGIF the width of the protein bands is practically independent of the protein concentration, the variation in peak area is accurately determined by the peak heights.

With the selected staining procedure the optical density of the specific protein bands of beef and pork show a linear response in function of the percentage composition. Fig. 2 demonstrates that, although the absorption of the selected protein bands differ widely at a given dilution, the slopes of the curves are rather similar.

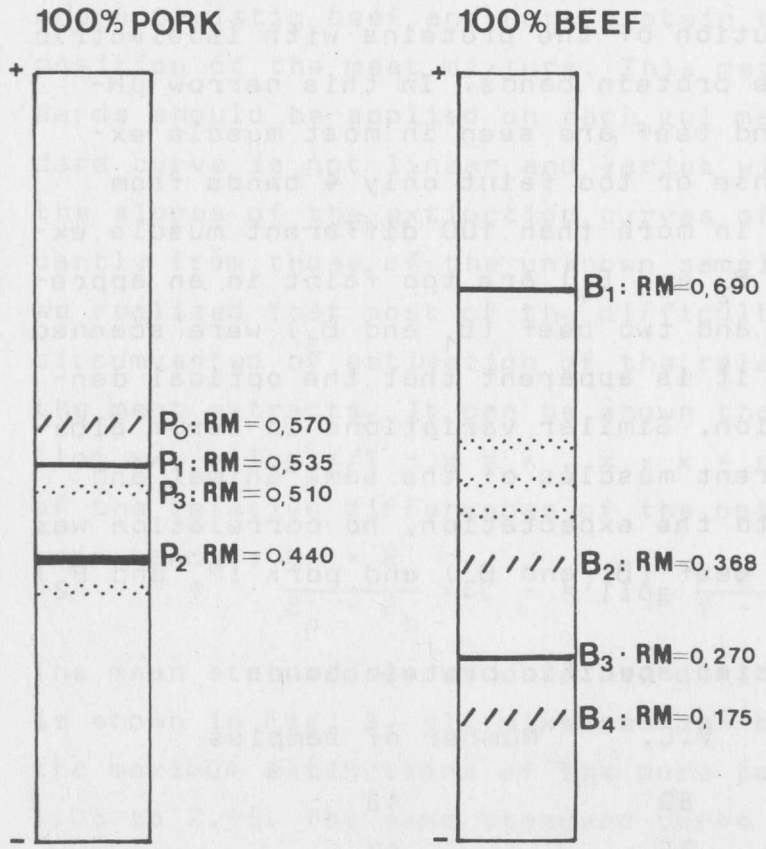

Fig. 1. RM-values of some specific protein bands of pork and beef in PAGIF pH 5.0 - 8.0

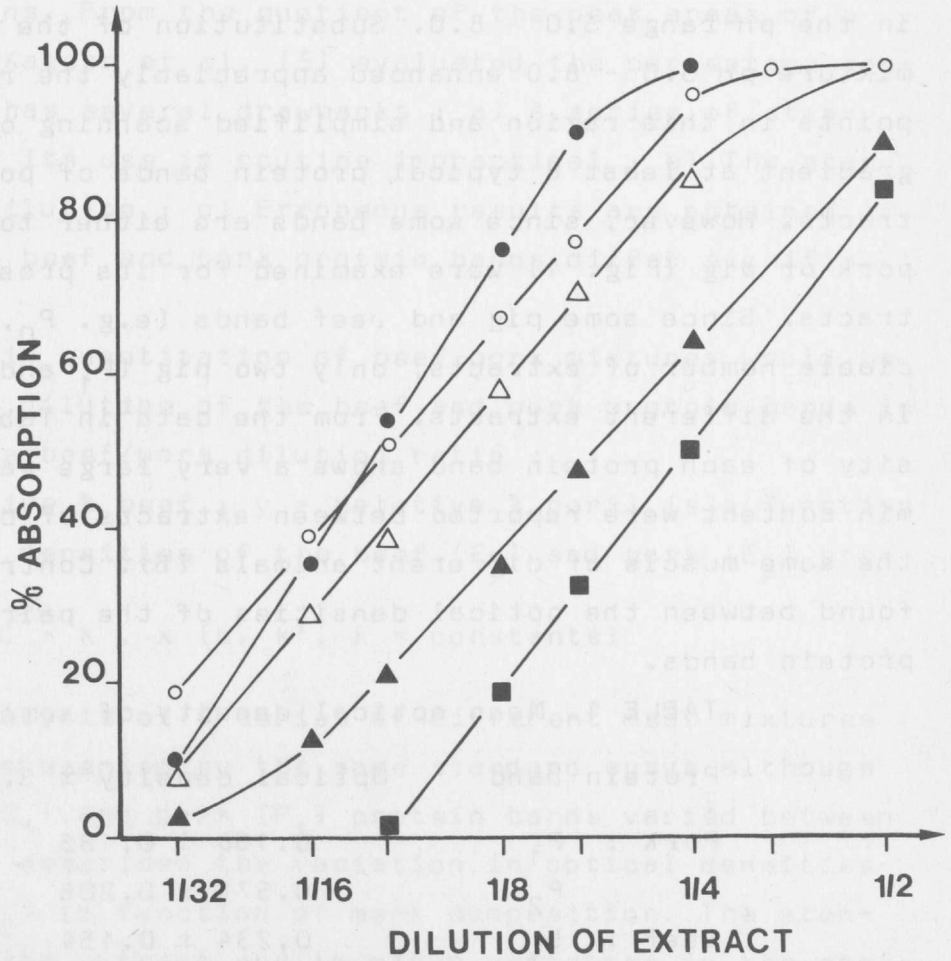

Fig. 2. Variation of absorption of some species specific protein bands with dilution Pork P_1 (○), P_2 (●), P_3 (▲) Beef B_1 (■), B_2 (△), B_3 (△)

2. Variations in the sarcoplasmatic protein patterns of beef and pork muscle extracts :
Preliminary experiments on pH 3.5 - 9 agarose gels indicated that most species specific protein bands of animal species of interest (pork, beef, sheep, horse, hen) are localized in the pH-range 5.0 - 8.0. Substitution of the pH 3.5 - 9 LKB ampholine buffer for the mixture pH 5.0 - 8.0 enhanced appreciably the resolution of the proteins with isoelectric points in this region and simplified scanning of the protein bands. In this narrow pH-gradient at least 8 typical protein bands of pork and beef are seen in most muscle extracts. However, since some bands are either too dense or too faint only 4 bands from pork or pig (Fig. 1) were examined for its presence in more than 100 different muscle extracts. Since some pig and beef bands (e.g. P_0, P_3, B_2 and B_4) are too faint in an appreciable number of extracts, only two pig (P_1 and P_2) and two beef (B_1 and B_3) were scanned in the different extracts. From the data in Table 1 it is apparent that the optical density of each protein band shows a very large variation. Similar variations in serum albumin content were reported between extracts of different muscles of the same animal and the same muscle of different animals (6). Contrary to the expectation, no correlation was found between the optical densities of the pairs of beef (B_1 and B_3) and pork (P_1 and P_2) protein bands.

TABLE 1. Mean optical density of some species specific protein bands

Protein band		Optical density ± S.E.	V.C.	Number of samples
Pork :	P_1	0.168 ± 0.135	80	43
	P_2	0.576 ± 0.206	36	43
Beef :	B_1	0.234 ± 0.154	66	47
	B_3	0.516 ± 0.318	62	47

3. Quantitative estimation of beef-pork mixtures :

The composition of a meat mixture can be estimated semi-quantitatively by visual comparison of the intensity of the species specific protein bands with that of a series of beef-pork mixtures run under identical conditions. From the quotient of the peak areas of a characteristic beef and pork protein band Kaiser et al. (5) evaluated the percentage composition of the meat mixture. This method has several drawbacks : a) A series of standards should be applied on each gel making its use in routine impractical ; b) The standard curve is not linear and varies with dilution ; c) Erroneous results are obtained if the slopes of the extinction curves of the beef and pork protein bands differ significantly from those of the unknown samples.

We realized that most of the difficulties in quantitation of beef-pork mixtures would be circumvented by estimation of the relative dilution of the beef and pork protein bands in the meat extracts. It can be shown that the beef/pork dilution ratio :

(log x/y = log x/1 - x $\simeq$ k . x ; x = relative % beef ; y = relative % pork) is a function of the relative differences of the optical densities of the beef (E_b) and pork (E_p) protein bands : $\frac{E_b - E_p}{E_p - E_b} \simeq C - k'.\log \frac{x}{1 - x} = C - k \cdot x$ (c, k', k = constants)

The mean standard curve obtained during analysis of 9 series of different meat mixtures is shown in Fig. 3. All mixtures can be represented by the same standard curve although the maximum extinctions of the pure beef (B_1) and pork (P_1) protein bands varied between 1.06 to 2.15. The same standard curve also described the variation in optical densities of another pair of protein bands (B_3 and P_2) in function of meat composition. The standard curve was independent of dilution of the extract and to minor variances in the staining procedure. The standard error, calculated from regression analysis, amounts to 2.75 %.

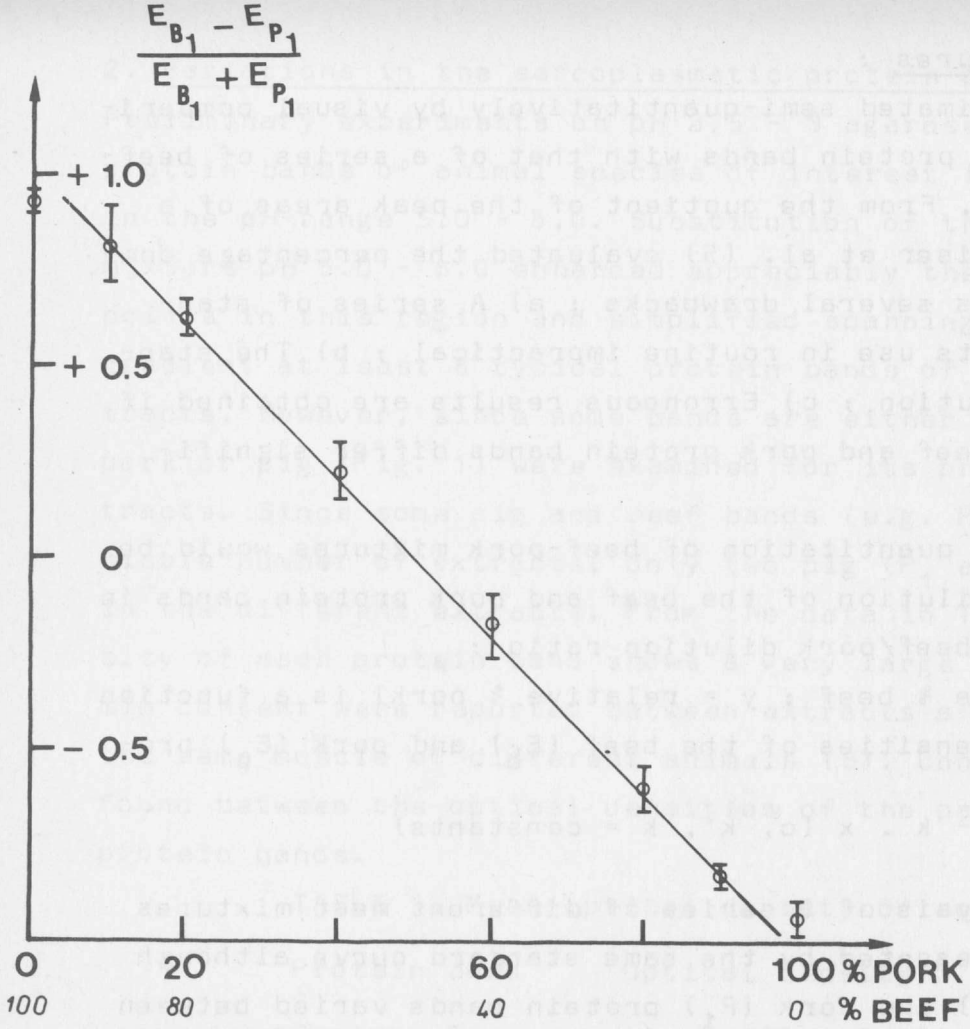

Fig. 3. Relationship between percentage composition of beef-pork mixtures and relative difference in optical density (each point is the mean of 9 determinations ± S.E.)

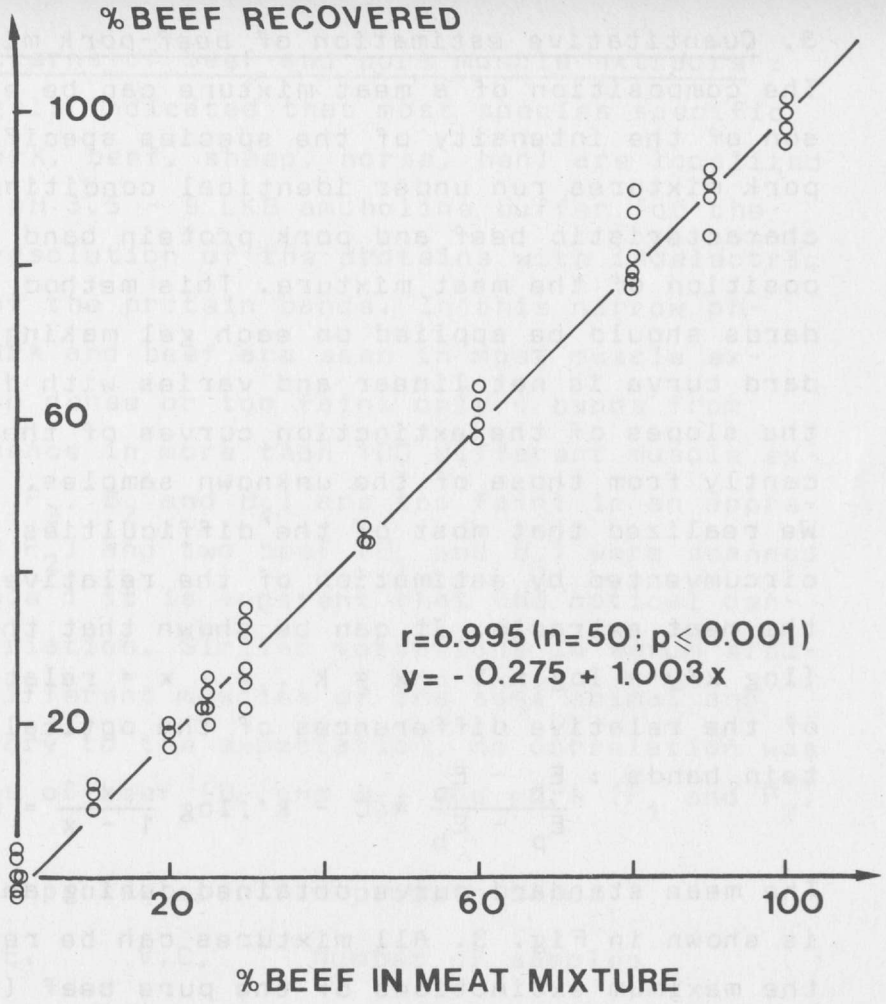

Fig. 4. Comparison of experimentally determined beef-pork composition with calculated values in 50 different samples using the difference in O.D. of two pairs of protein bands. The solid line was determined by regression analysis.

4. Application of the method to beef-pork mixtures :

Fifty different beef-pork mixtures were prepared. From each meat extract three dilutions (1/4, 1/8, 1/16) were applied on the gel (see materials & methods). The different dilutions allow selection of the optimal optical density (O.D.) of the pairs of protein bands (P_1, B_1 and P_2, B_3). Following densitometry, the relative differences in O.D. for the two pairs of protein bands were calculated at the appriopriate dilutions. The percentage composition of the meat mixture was evaluated from the mean standard curve (Fig. 3). The results obtained on 50 different samples are represented in Fig. 4. The results show that this method allows an accurate determination (V.C. 4.5 %) of the percentage composition of beef-pork mixtures.

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