Note

Identification of Sea Turtle Meat Species by Thin Layer Polyacrylamide Gel Isoelectric Focusing

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Thin layer polyacrylamide gel isoelectric focusing provides very rapid and reliable method for identification of sea turtle meat species (green turtle) , (Chelonia mydas), loggerhead (Caretta caretta), olive ridley (Lepidochelys olivacea) , hawksbill (Eretmochelys imbricata), and leatherback (Dermochelys coriacea) . The proteins in the meat extracts gave specific patterns in a pH—gradient of 5—8 after staining of the gel by Coomassie R—350. It was also confirmed that sea turtle meat species were easily distinguishable from other meat species by this method.

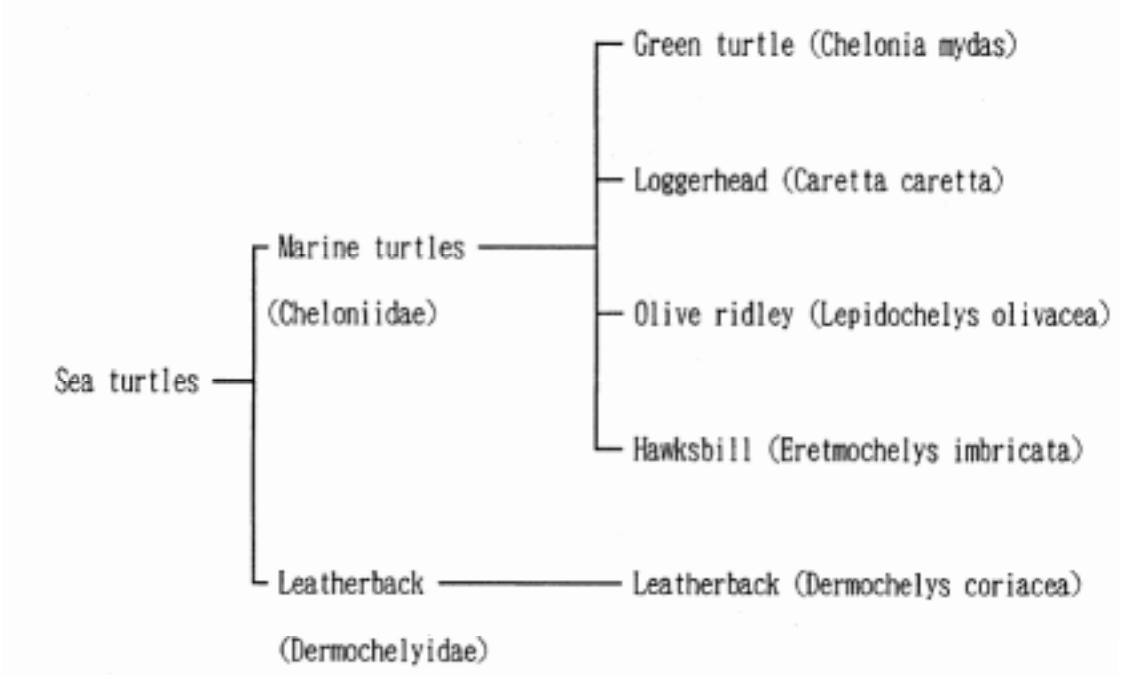
INTRODUCTION

The Official Methods of Analysis of AOAC〔1〕 prescribe the method for identification of fish meat species by isoelectric focusing (IEF), whereas Yman reported on applications of lEF for identification of edible meat species〔2〕. Suzuki et al. examined identification method for cooked beef and pork by IEF〔3, 4〕. Proteins in meat extract focusing at the its isoelectric point produce a protein pattern specific to the species of meat, sometimes called a " fingerprint " of the species〔2〕.

IEF was performed in order to certify that this method could be applicable to identify sea turtle meat species and to differentiate them from other meat species. Possible problems concerning identification of unknown meat species by IEF method were also examined.

It is very difficult to identify meat species from their appearance, texture, smell or by other physical examinations, if meat is presented without a trace of turtle skin or shell. However, identification of sea turtle meat species is considered to be important because international trade in these turtles is prohibited or strictly restricted by the Convention on International Trade in Endangered Species of Wild Fauna and Flora. On the other hand, meat of certain sea turtle (e. g., green turtle) is sometimes illegally traded for the purpose of human consumption. As shown in Table 1, sea turtles are divided into two families ; i. e., marine turtle family (Cheloniidae) and leatherback family(Dermochelyidae) . The Marine turtle family is considered to consist of four species, i. e., green turtle (Chelonia mydas) ,

Table 1　SEA TURTLES



loggerhead (Caretta caretta) , olive ridley (Lepido—chelys olivacea) and hawksbill (Eretmochelys imbricata) , whereas the leatherback family has only one species (leatherback, Dermochelys coriacea).

EXPERIMENTAL

Materials

Authentic frozen shoulder meats of green turtle (Chelonia mydas) , loggerhead (Caretta caretta) , olive ridley (Lepidochelys olivacea) , hawksbill (Eretmochelys imbricata) , and leatherback (Der—mochelys coriacea) were supplied by Port of Nagoya Public Aquarium. Authentic frozen bryde's whale meat (Balaenoptera edeni) was obtained from the National Research Institute of Far Seas Fisheries. Beef and pork was purchased from the market. Frozen harp seal (Pagophilus groenlandicus) meat was supplied by Toyotsu Busshi Hanbai Co. Unknown frozen meat samples seized by the Japanese Customs were also analyzed.

Preparation of samples

About 1g of each sample were thawed, This drip is filtered through Toyo No.1 filtering paper and diluted by ion exchange water 2—4 times.

Isoelectric focusing

IEF was carried out in the PhastSystem (Pharm acia, Uppsala, Sweden) on PhastGel lEF3—9 using the recommended procedure〔5〕: pre—focusing at2,000V, 2.5mA, 3.5W (limiting) for 75Vh ; 0.5μl of sample per lane was applied automatically at 200V 2.5mA 3.5W 15Vh ; final focusing at 2,000V, 2.5mA, 3.5W, 410Vh (these focusing process took ca. 30min) . The gel was stained automatically with PhastGel Blue R(Coomassie R — 350) in the Development Unit of the PhastSystem according to the recommendations given in the operating manual〔6〕 (fixing : 20℃, 5min, st aining : 50℃, 10min, destaining : 50℃, 10min) .The focused bands were compared with the positions of simultaneously focused proteins of Broad pI Calibration Kit (pH3 — —10) (purchased from

Phamacia) in order to determine pI values. Densitograms of IEF protein patterns of the meat extracts from the samples were obtained with the UltroScan XI (Phrmacia).

RESULTS AND DISCUSSION

IEF was performed in thin layer polyacrylamide gels in a pH3—9 gradient.

The proteins in meat extracts were focused at the pH corresponding to their isoelectric point. All isoelectric focusing protein patterns had the bands at pI 5 where samples were applied. Employing this method, it took only less than 90 minutes from sample preparation to identification of meat species.

Since the protein patterns of sea turtle meat extracts were completely different from those of beef, pork, whale meat and seal meat(Fig.1, 2 and 3), it was confirmed that sea turtle meat species were clearly distinguishable from other meat species by this method.

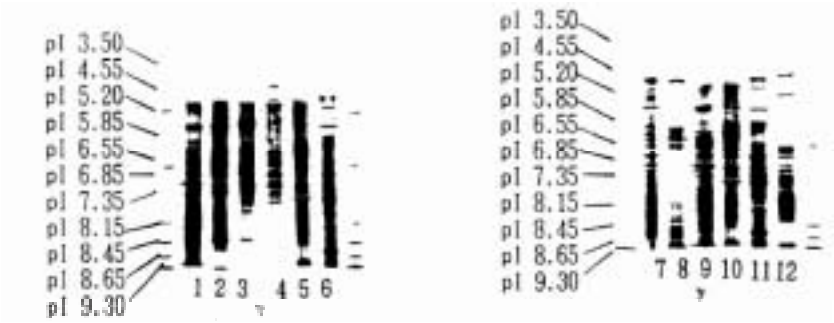


Fig. 1 Isoelectric focusing of meat extracts : 1. Green turtle (Chelonia mydas) ; 2. Loggerhead (Caretta caretta) ; 3. Olive ridley (Lepidochelys olivacea) ; 4. Hawksbill (Eretmochelys imbricata) ; 5. Le atherback (Dermochelys coriacea) ; 6. Bryde's whale (Balaenoptera edeni) ; 7. beef ; 8. pork ; 9. Green turtle ; 10. Leatherback ; 11. Bryde's whale ; 12. Harp seal (Pagophilus groenlandicus) ; Protein patterns of both side of each gel are those of Broad pI Calibration Kit (pH3—10) (Pharmacia).

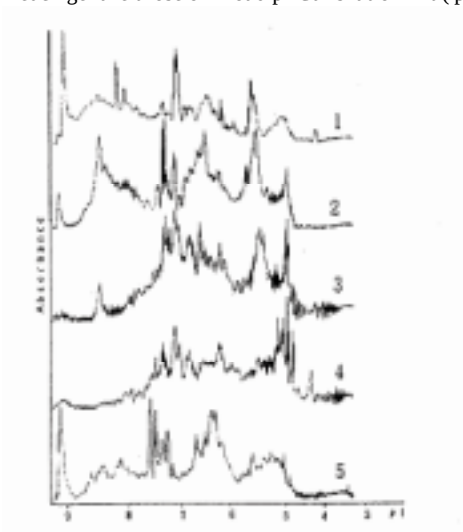


Fig. 2 Densitograms of isoelectric focusing of sea turtle meat extracts : 1. Green turtle ; 2.Loggerhead ; 3. Oive ridley ; 4. Hawksbill ; 5. Leatherback

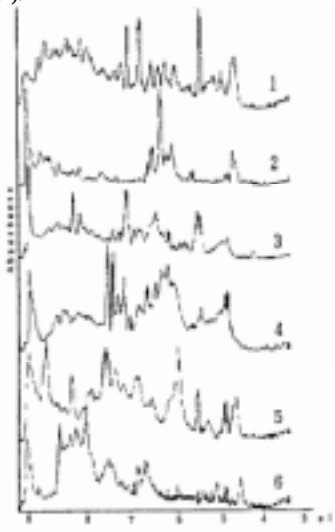


Fig. 3 Densitograms of isoelectric focusing of various meat extracts : 1. beef ; 2. pork ; 3. Green turtle ; 4. Leatherback ; 5. Bryde's whale ; 6. Harp seal

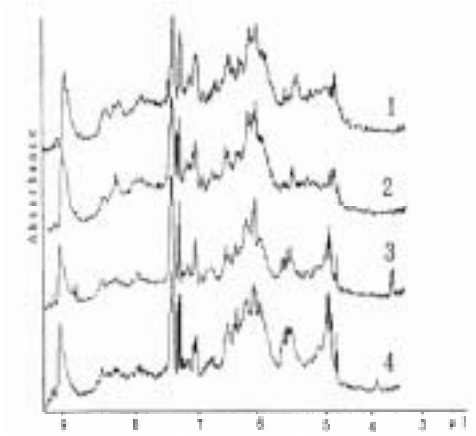


Fig. 4　Densitograms of isoelectric focusing of meat extracts of unknown samples (1. 2. and 3.) and authentic leatherback (4 .)

As shown in Fig .1 and 2, the protein patterns of marine turtles showed more or less similar patterns. On the other hand, the protein pattern of leatherback were not similar to those of marine turtles. In particular, the several bands around pI 7.5 in the leatherback sample were characteristic of this species, whereas similar bands in those of marine turtle species were located around pI 7. Hawksbill was distinguishable from other marine turtle species on the grounds that its protein pattern lacked a clear band around pI 6.5 whereas other marine turtles showed strong bands in this region. Green turtle had only one clear protein band near pI 7 Whereas a couple of strong bands were observed in protein patterns of loggerhead and olieve ridley in this pI region. Loggerhead and ridley showed very similar protein patterns, although they were distinguishable because Loggerhead had a strong broad band between pI 6—7 whereas olieve ridley had separate several bands.

IEF was performed for unknown samples seized by the Japanese Customs, and these samples were turned out to be leatherback meat (Fig.4) . Strong four protein bands between pI 7—7.5 appeared in IEF pattern of unknown 3 samples were almost identica1 to those of the authentic leatherback meat. Furthermore, other part of protein patterns of these unknown samples and the authentic meat were very similar, although small discrepancies were observed between protein patterns of those samples and authentic meat. This discrepancy might be attributed to the difference of parts of meat taken or difference of individuals. However, it was impossible to carry out further examinations because of the difficulty of obtainment of other authentic meats of different parts or different individuals. In spite of this fact, it was appeared that sea turtle meat species could be easily identified by this method in comparing protein patterns of samples with those of simultaneously focused authentic samples.

ACKNOWLEDGMENTS

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