Journal for the Improvement of Animal Husbandry

UDC636 **ISSN 1450-9156**

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

9 th INTERNATIONAL SYMPOSIUM
Modern Trends in Livestock Production

BELGRADE - SERBIA
7 – 9 October 2009

Belgrade - Zemun 2009

Biotechnology in Animal Husbandry 25 (5-6), p 895-899, 2009
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.5'62

QUALITATIVE DETERMINATION OF PORK SPECIES CONTENT IN COOKED MEATS USING ENZYME IMMUNOASSAY TECHNIQUES: CASE BEEF JERKY AND SHREDDED MEAT PRODUCTS IN WEST JAVA – INDONESIA

R. L. Balia[1], H. A. W. Lengkey[1], S. Mudjiartiningsih[2], E. Rosiannie[2], L. Suryaningsih[1], W. P. Setyadi[1], A. R. Ghani[3], L. U. S. Gemilang[3]

[1]Faculty of Animal Husbandry, Padjadjaran University, Bandung, Indonesia
[2]Veterinary Public Health and Animal Diseases Investigation Center, Lembang, Indonesia
[3]Institute of Ecology, Padjadjaran University, Bandung, Indonesia
Corresponding author: roostita@gmail.com
Original scientific paper

Abstract: This work is aimed to determine pork species content in cooked meats which using enzyme immunoassay techniques. Twenty four cooked meats samples were taken from 3 regions in West Java province; there are Cianjur (nine beef jerky samples), Cimahi (eight beef jerky samples) and Bogor (seven shredded meat samples). The result showed that two from nine beef jerky sample from Cianjur (Brenggolo Jerky and ACC Beef Jerky) and one from eight beef jerky samples from Cimahi ("Cap Kepala Sapi" Jerky) were shown positive containing pork meat species as indicated as adulteration in beef jerky products.

Key words: species content, cooked meats, enzyme immunoassay techniques

Introduction

Ingredients substitution was frequently found in meat products such as in beef jerky and shredded meat as adulterations. Ingredients substitution was relatively not easy to recognize because raw muscle tissue from different species is often similar in appearance once incorporated in a comminuted product. In some cases, substitution of declared or implied meat product ingredients with cheaper ingredients is commonly found. The cheaper ingredients are either from the species declared (typically offal, blood, connective tissue) or meat which came from different species.

Ingredients substitution practices break no laws but in the longer term it intent to condemn meat into low price. On the other hand, in many countries

ingredients of meat product were monitored. Ingredients substitution can also generate religious concern, such as pork contains ingredients are prohibited by Moslems (non-halal).

Religious strictures, perceived or real health concerns and cultural likes and dislikes are the main drivers of species identification for consumer protection. All methods to determine species are based on biochemistry in one form or another because qualitative and quantitative biochemical traits set all individuals apart from one another.

Main method for species identification on 1990s period was based on the properties of the proteins *(Barai et al., 1992)*. In these techniques, proteins are extracted from beef jerky or shredded meat with solvents and subjected to electrophoresis or immunological analysis. Typical immunological methods rely on diffusing the extracted proteins of interest against antibodies to proteins from a range of different animal or plant species.

Adulteration test on meat product were so difficult, especially heat processed product like beef jerky and shredded meat, heat process resulting denaturized proteins *(Hoffman 1996)*. Therefore, antibodies to heat-stable soluble proteins, which retain their antigenicity after high temperature process, must be prepared *(Rencova et al., 2000)*.

The aim of the research was to determine pork species content in cooked meats which using enzyme immunoassay techniques (EIA), hence it could prevent the substitution of meat destined for human consumption with unsuitable species of meat.

Materials and Methods

Twenty four samples taken from 3 regions in West Java, Cianjur (nine beef jerky), Cimahi (eight beef jerky) and Bogor (seven shredded meat). These samples are prepared for enzyme immunoassay test using Tepnel BioKits Pork Cooked Identification Test Kit Cat. No.902012N.

Samples preparation. Twenty five gram samples (beef jerky or shredded meat) minced and blended and then put into a stomacher bag. Add 100mL of saline water, stomach on medium setting (230 rpm) for 2 minutes. Transfer contents to a microwavable container and heal on full power for 90 seconds, allow standing for 15 minutes. Transfer mixture to clean stomacher bag and stomach for further 2 minutes, allow standing for 15 minutes. An amount of liquid should appear above a settled layer; filter the liquid through whatman no.4 or similar filter paper into clean container; alternatively a slurry may be obtained which may require centrifugation to obtain a suitable extract. Mix the sample extract, which is now ready for immunoassay.

Enzyme immunoassay technique. Pipette 100µl sample extract or positive control into respective test wells. Incubate at room temperature for 45 minutes. Wash wells three times with working wash solution. Dispense 50µl anti-species biotinylate into all test wells and then incubate at room temperature (18-22˚C) for 45 minutes. Wash wells three times with working wash solution. Dispense avidin peroxidase conjugate into all test wells. Incubate at room temperature (18-22˚C) for 15 minutes. Wash wells five times with working wash solution. Dispense 100µl TMB substrate solution into each test wells. Incubate at room temperature (18-22˚C) for 45 minutes. Dispense 50µl stop solution into each assay well and mix plate. Measure absorbance (450nm) of each assay well or well contents using plate render or spectrophotometer or visually assess plate.

The kit was utilizing a biotin-avidin enhancement process. With increased concentrations of pork-specific protein in the extract, more of the protein will bind to antibody attached to the well. After allowing the reaction to proceed, unbound material is removed by washing. The amount of specific protein bound to the antibody coated well is determined by reaction firstly with biotinylated and also with a streptavidin-peroxidase conjugate. After incubation, access reagent is removed by washing. Finally, bound peroxidase activity is determined by adding a fixed amount of TMB substrate which develops a blue color (changing color to yellowish green on addition of acid stop reagent) in the presence if peroxidase. Color development is proportional to the original amount of specific pork protein in the samples extract.

Results and Disscussion

Twenty four samples was tested using enzyme immunoassay technique. The test was conducted at room temperature of 18-22˚C. Under these conditions negative samples wells should appear virtually colorless to the naked eyes, while positive samples will give yellowish green coloration as shown in Figure 1 lines A4, C4 and F2. Each positive control is equivalent to sample containing significant level of pork meat.

Pork specific antibody that contain on samples were linked with the peroxidase and then showing yellowish green color on tests well. While, other samples with non-pork (beef) specific antibody were not showing color reaction. The color of positive samples produced by enzyme –peroxidase– which covalently linked to the antibody that amplifies the precipitin complex by generating many more chemical equivalents of (colored) reaction product than are inherent in the precipitin complex *(Hui et al., 2001)*. Enzyme conjugates an appropriate secondary antibody followed by enzyme catalyzed color reaction *(Walker, 2002)*.

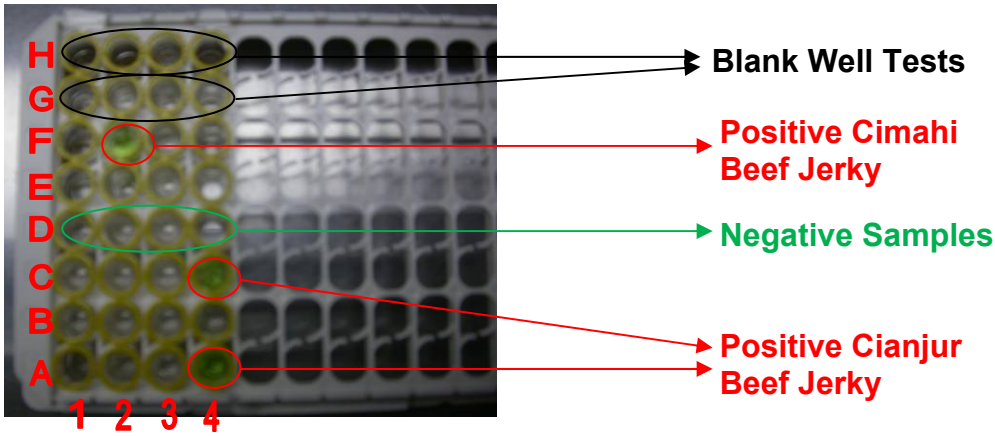

Figure 1. Enzyme immunoassay result

Conclusion

Determination of pork species content in cooked meat products such as beef jerky and shredded meat could be done which using enzyme immunoassay technique. Beef jerky samples that are positively adulterated by pork species content shown as yellowish green color as shown in Figure 1 lines A4, C4, and F2. Enzyme immunoassay technique can be use to determine adulteration practice on meat products that processed with high temperature.

Kvalitativno određivanje sadržaja svinjskog mesa u kuvanim mesnim proizvodima korišćenjem imuno analize enzima: proizvodi od govedine i mesni proizvodi od seckanog mesa u Zapadnoj Javi, Indonezija

R. L. Balia, H. A. W. Lengkey, S. Mudjiartiningsih, E. Rosiannie, L. Suryaningsih, W. P. Setyadi, A. R. Ghani, L. U. S. Gemilang

Rezime

Cilj ovog istraživanja je bio utvrđivanje sadržaja svinjskog mesa u kuvanim mesima korišćenjem tehnika imuno analize enzima. 24 uzorka kuvanih mesnih proizvoda su uzeti iz 3 regiona u provinciji Zapadna Java; Cianjur (devet uzoraka govedjih proizvoda), Cimahi (osam uzoraka govedjih proizvoda) i Bogor (sedam uzoraka mlevenog mesa). Rezultati su pokazali da su 2 od devet goveđih

proizvoda iz Cianjur-a (Brenggolo Jerky i ACC Beef Jerky) i jedan of osam uzoraka iz regiona Cimahi ("Cap Kepala Sapi" Jerky) imali pozitivne rezultate prisustva svinjskog mesa što se smatra kvarenjem, krivotvorenjem goveđih mesnih proizvoda.

References

BARAI B.K., NAYAK R.R., SINGHAL R.S., KULKARNI P.R. (1992): Approaches to the Detection of Meat Adulteration. Trends. Food.Sci. Technol., 3, 69-72.

HOFFMAN K., FISHER K., MILLER E., KEMPER V. (1996): Experiments to Demonstrate the Effectiveness of Heat Treatments Applied to Canned Meats and Meat-and-Bone Meals. Fleishwirshaft, 76, 920-923.

RENCOVA E., SVOBODA I., NECIDOVA L. (2000): Identification by ELISA of Polutry, Horse, Kangaroo and Rat Muscle Specific Proteins in Heat-Processed Products. Vet. Med. Czech, 45, 12, 353-356.

HUI Y.H., WAI-KIT N., ROGERS R.W., YOUNG O.A. 2001. Meat Science and Applications. Marcel Dekker, Inc. New York – Basel.

WALKER J.M. (2002): The Protein Protocols Handbook. Second Edition. Humana Press.

Received 31 May 2009; accepted for publication 15 August 2009