DETECTION OF PORK SPECIES CONTENT ON BEEF JERKY AND SHREDDED MEAT BY USING ENZYME IMMUNOASSAY TECHNIQUES

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The identification of pork species content in beef jerky and shredded meat was performed for various reasons, both economics and ethnic. The aims of identification were to prevent the substitution of meat destined for human consumption with unsuitable species or inferior kind of meat. The research was held at 3 regions in West Java Province such as Depok city (nine beef jerky samples), West Bandung district (five beef jerky and four shredded meat samples) and Bandung district (six beef jerky and one shredded meat) that twenty-five samples have taken. Result showed that two from nine beef jerky samples from Depok (Kitiran Jerky, SP.0079/13.06/90) were positive in pork species content as indicated as adulteration in beef jerky products. The research showed that uses of enzyme immunoassay technique were available for consumer protection and meat safety from products adulteration.

Key words: Pork Species Content, Beef Jerky, Enzyme Immunoassay Techniques

Adulterations with ingredients substitution way were frequently found in meat products such as beef jerky and shredded meat. Ingredients substitution was relatively easy because raw muscle tissue from different species is often similar in appearance and once incorporated in a comminuted product. At another level, substitution of declared or implied meat product ingredients with cheaper ingredients is very common. The cheaper ingredients are either from the species declared (typically offal, blood, connective tissue) or meat which came from different species.

Sometimes these practices break no laws but in the longer term it intent to condemn meat into low price and make more money. In many countries, ingredients of meat product were monitored. Ingredients substitution can also generate religious concerns: pork contains ingredients use or consumption was prohibited in Moslems Countries.

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.

The difficulties in meat product adulteration test, 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 objective of the research was to detect pork species specific content on heated process meat products such as beef jerky, shredded meat with using enzyme immunoassay technique, hence it could prevent the substitution of meat destined for human consumption with unsuitable species of meat.
MATERIAL AND METHOD

Twenty five samples taken from 3 regions in West Java Province such as Depok city (nine beef jerky samples), West Bandung district (five beef jerky and four shredded meat samples) and Bandung district (six beef jerky and one shredded meat). After that, samples prepared for enzyme immunoassay test which using Tepnel BioKits Pork Cooked Identification Test Kit Cat. No.902012N.

Samples Preparation

Twenty five gram samples from each of beef jerky or shredded meat blended, minced or finely chopped and 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 heat 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 reader or spectrophotometer or visually assess plate.

RESULT AND DISCUSSION

![Figure 1. Enzyme Immunoassay Result](image)

- Blank Well Tests
- Positive Samples
- Negative Control
- Positive Control
Twenty five samples was tested using enzyme immunoassay technique. The test was done at room temperature of 18-22°C. Under these conditions negative control wells (A2-A4) should appear virtually colorless to the naked eyes, while positive controls (A1) and positive samples (C1, F3) will give medium yellow coloration. Each positive control is equivalent to sample containing significant level of pork meat.

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 yellow 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.

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).

CONCLUSIONS

Enzyme immunoassay technique was effective to detect species specific content on heated process meat products such as beef jerky and shredded meat. Two beef jerky samples positive adulterated by pork meat, it is visible on medium yellow coloration of well test kit (C1, F3).

REFERENCES