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# DETERMINATION OF RAT ADULTERATION IN MEATBALLS USING ENZYME - LINKED IMMUNOSORBENT ASSAY (ELISA) TECHNIQUES AT JATINANGOR EDUCATION AREA, SUMEDANG DISTRICT, WEST JAVA, INDONESIA

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***Abstract***

*Currently, one of the most significant threats is the excessive hunting on wild animals. This is because the hunting results into a food product with turnover and big profit. The trigger for the demand for animal protein derived from the flesh of wild animals (bush meat) against certain species may lead to an increase in diseases. Adulteration processed meatballs into one type of processed replaces raw materials with rat meat. In addition, to causing economic losses, the food products consumed are not safe, healthy, whole, and halal, therefore the need for identification of these food products appears. One method that can be used to detect food product adulteration is the ELISA (Enzyme-Linked Immunosorbent Assay). This test method is an effort to detect the presence of antibodies or specific antigen in a sample. A total of 29 samples were collected from various meatball traders and chicken noodles around Jatinangor. The results showed negative results of 96.55% and positive results 3.45%. A positive result has a greater value than a negative result color (blue) indicating that rat antibody samples bind to streptavidin-peroxidase antigens, where antigen and antibodies occur in homologous process resulting in a change of color (yellow). This test has a high sensitivity level, so that on products that have experienced ripening can still be detected specific antigen. This is evidenced in the positive control of rat meatballs, and antibodies in rat can still be detected. The negative results indicate there is no adulteration meat meatball in the meatball and it is an evidence that Halal in the area of Jatinangor and surrounding areas become an important concern on food in the campus environment.*

***Key words****: adulteration, ELISA, rat meat.*

## INTRODUCTION

Adulteration food processed products from meat is very frequent. Raw materials are replaced with other materials, that are cheaper. In 2013 in the United Kingdom a lot of processed beef products are substituted with horse or pork. One example is the beef burger products, where beef is replaced with horse meat. In China, rat and fox meat were sold as lamb. The meat is processed by adding gelatin, nitrate salts and stains, so it looks and feels like frozen goat meat. In 2017, in some areas in Indonesia are found several cases of counterfeiting cases on beef meatball products and chicken noodles. The products are substituted with pork or rats (Fumière et al., 2009).

Food products with processed rat meat can cause various losses for consumers. In addition to causing economic losses, the products consumed become not safe, healthy, whole, and halal. Foods faked with rat meat have the potential to cause various diseases.

This is related to the potential diseases or parasites found in rat meat. Research from the World Health Organization found that 1 in 10 people in the world suffer from diseases caused by the food they eat, 420,000 of whom die every year, many of them children.

Several methods have been developed based on electrophoresis, isoelectric focus, chromatography, DNA hybridization, polymerase chain reaction (PCR) and enzyme-linked immunesorbent assay (ELISA) (Aida et al., 2005).

Therefore, it is necessary to identify the falsification of processed foods. The falsification test on meat products is very difficult, especially heating processed products such as meatballs, because the heating process produces a denaturative protein. Antibodies to proteins

dissolve in a stable heat, which retains its antigenicity after high temperature processes (Olivier et al., 2009). One method that can be used to detect food product counterfeiting is the ELISA (Enzyme-Linked Immunosorbent Assay) method. This test method is an effort to detect the presence of antibodies or specific antigen in a sample (Ayaz et al., 2006).

The ELISA approach allows the identification of various types of meat mixtures in very low quantities or has undergone changes caused by processing. The test is expected to provide guarantee and guarantee of quality and safety of processed products (meatballs) produced in Jatinangor Sumedang, West Java-Indonesia.

## MATERIALS AND METHODS

A number of antigens / antibodies are affixed to a surface (well), then added substances that can be converted by the enzyme into a detectable signal. A total of 29 samples were collected from various meatball traders and chicken noodles around Jatinangor.

The samples will be tested using the ELISA method with the Cat # RMT-48 Cat Test Kit from Alpha Diagnostic International.

**Sample Preparation (extraction)**

Samples are cut into small pieces, and mixed until homogeneous (in the code of the same sample). It is putted into tube test as much as 100 mg. The buffer is diluted 100x. Add the extraction buffer according to the instruction kit (incubation 4˚C ± 24 hours). Positive controls were prepared (concentration of raw meatball 10%, 20%, 30%, 40%, 50%, concentration of meatball cooked 10%, 20%, 30%, 40%, 50%).

**ELISA Test**

The microtiteris marked on the plate. The supernatant of sample extraction result ± 1 ml prepared. 100 µl positive and negative controls are added into a well. 100 µl of sample extraction are addedinto other wells. It is stired, by tapping well for 5-10 seconds. It is incubated at room temperature for 30 minutes. It is disposed of well contents (washed) and washed with wash buffer 3x (buffer used as much as 300µl).

It is added 100 µl antibody-enzyme conjugate into each well. It is stired, by tapping well for 5-10 seconds. It is incubated in room temperature for 20 minutes. It is discarded the contents well (spilled) and washed with the wash buffer as much as 4x (buffer used as much as 300 µl). It is added 100 µl TMB Substrate. It is stired, by tapping well for 5-10 seconds. Ii is incubated in room temperature for 10 minutes (well will be blue). It is added Stop Solution as much as 100µl (positive well will turn to yellow).

It is uppered on ELISA reader with wavelength 450 nm.

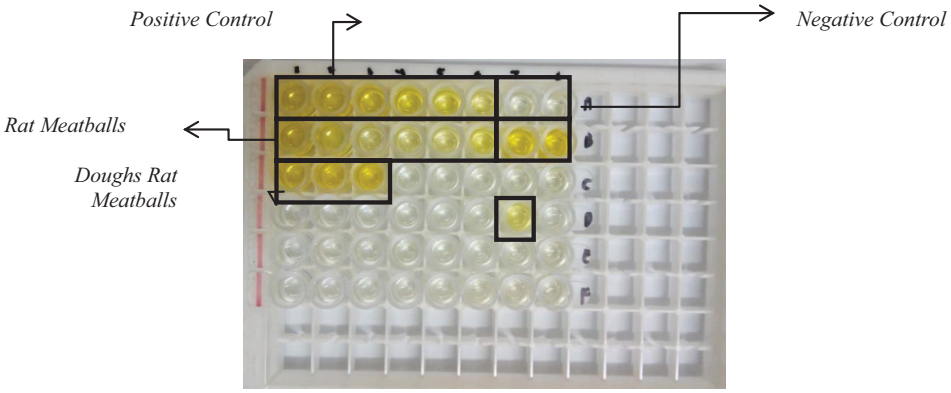


Figure 1. Result Enzyme immunoassay

## RESULTS AND DISCUSSIONS

ELISA is used in order to test screening (screening test), to detect protein levels in the presence of antigens and antibodies. ELISA tests are not only sensitive, but also rapid and specific, because of complex antibodies that occur in well microplate and after substrate administration, enzymes bound to antibodies

will give color change to the fluid, giving different optical density.

Twenty-nine samples were taken from Jatinangor meatballs traders to identify adulteration on meatballs sold and tested using ELISA. The ELISA kit uses an improved biotin-avidin process. With the increase in the specific protein concentration of rat meat in the extract, more proteins bind antibodies attached to well.

After the reaction process continues, the unbound material is removed by washing. The amount of specific proteins attached to well-coated antibodies is determined by the first reaction with biotinylation and also with the conjugate streptavidin-peroxidase. After incubation, the reagents are removed by washing. Finally, the bound peroxidase activity is determined by adding a certain amount of Tetramethylbenzidine (TMB) substrate, which develops blue color (turns yellowish green with the addition of acidic reagents) in the presence of peroxidase. The development of color is proportional to the original number of certain rat proteins in the sample extract.

| R/C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| A | 2.238 | 2.210 | 1.928 | 1.380 | 0.863 | 0.549 | 0.177 | 0.180 |
| B | 2.382 | 0.902 | 0.397 | 0.414 | 0.439 | 0.724 | 2.619 | 2.792 |
| C | 2.395 | 2.437 | 2.638 | 0.116 | 0.132 | 0.127 | 0.135 | 0.163 |
| D | 0.095 | 0.097 | 0.095 | 0.114 | 0.099 | 0.108 | 0.546 | 0.109 |
| E | 0.112 | 0.124 | 0.111 | 0.127 | 0.104 | 0.107 | 0.127 | 0.150 |
| F | 0.108 | 0.106 | 0.113 | 0.108 | 0.129 | 0.130 | 0.130 | 0.122 |

No. Sample B1-C3: Samples of processed rat meatballs, A1-A8: Control Samples, C4-F8: Meatballs Samples

: Negative : Positive

Figure 2. Result of ELISA Reader

At point D7 the sample has a greater value than the negative control it indicates that the rat antibody samples bind to the antigen streptavidin-peroxidase.

Where antigen and antibodies occur homologous process resulting in a change of color yellow from the process.

This test has a high sensitivity level so that on products that have experienced ripening can still be detected a specific antigen.

This is evidenced in the positive control of rat meatballs, antibodies in mice can still be detected.

This study shows that ELISA can identify the presence of rat meat in meatball dough and meatballs that have undergone a heating process. Chien et al. (2001) detected rat meat content in processed heating products by ELISA method with the lowest detection limit of 0.5% (b / w) of mice in the meat mixture. Roostita and Lengkey (2014) showed that the Enzyme-Linked Immunosorbent Assay (ELISA) check enables identification of various types of meat mixtures in very low quantities or has undergone changes caused by processing.

The test on 28 samples taken from meatballs merchants in Jatinangor showed no rat adulteration. This is because meatballs traders realize the importance of the quality of raw materials related to halal meatballs. Only 1 sample was found by traders to adulteration by mixing rat meat into meatballs, although at certain times it was profitable this can threaten the sustainability of the business. Traders already have knowledge about halal meatballs so that traders are not worried about the sale of meatballs. Halal is a sensitive issue because most of the population of Jatinangor are Muslim.

## CONCLUSIONS

The test ELISA tested 29 samples from traders meatballs and showed negative results of 96.55% and positive results 3.45%. The negative results indicate no adulteration rat meat. This is evidence that halal in the area of Jatinangor and surrounding areas become an important concern for traders and consumers in the campus environment.

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