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Development of a Homemade Sandwich ELISA Kit for Detection of Raw and Heated Rat Meat Extracts

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***Abstract*: This study focuses on the development of a homemade sandwich ELISA for the detection of rat meat components, employing locally produced polyclonal antibodies specific to rat meat as capture antibodies. These are paired with commercially available secondary antibodies for signal detection. The assay demonstrated the ability to identify the presence of raw rat meat and rat meat heated at 70°C for 15 minutes, with detection limits of up to 1:10,000 dilution (2.5 µg/mL) and 1:1,000 dilution (25 µg/mL), respectively. However, the assay was not able to detect rat meat heated at 100°C, likely due to heat-induced antigen denaturation. Despite this limitation, the ELISA system shows promise for further refinement and application in food authentication, particularly for detecting contamination from wild animal-derived ingredients such as rat meat, thereby contributing to food safety assurance and public health protection.**

***Keywords*: ELISA, food authentication, meat adulteration, rat meat.**

1. Introduction

Rats are members of the order Rodentia, a taxonomic group characterized by small mammals possessing continuously growing incisors adapted for gnawing. Within this order, rats are categorized into various species, with Rattus rattus [the black rat] being one of the most recognized. Another prominent species is the brown rat [Rattus norvegicus] [1]. In certain cultures, and geographic regions, the consumption of rat meat is customary. Typically, only specific species are selected, and the preparation methods follow traditional culinary practices [2]–[4]

However, public health concerns may arise when rat meat is inadequately cooked. This issue is particularly pronounced in densely populated urban environments, especially in informal settlements where rat populations thrive. In such areas, unscrupulous food vendors may incorporate rat meat into dishes like meatballs and noodles without disclosure. [1], [5]–[9] How rodents play a role in spreading foodborne diseases is discussed extensively by Hamidi [10].

Preventive efforts, such as educating communities on environmental hygiene and implementing proper cooking techniques to eliminate pathogenic microorganisms while preserving the nutritional value of food ingredients, have been conducted in various settings [11]. Besides that, in recent years, there has been significant growth in research focused on developing tools to verify the authenticity of healthy food products and ensure they are free from harmful biological contaminants. Among the commonly used detection methods is enzyme-linked immunosorbent assays [ELISA] [12], [13]. ELISA, that is highly specific against rat heat-resistant proteins have been reported by Chen et al. [14].

In several countries, ELISA technology remains relatively costly, which likely explains the continued efforts by many researchers to develop in-house or homemade ELISA systems [15]–[17]. These homemade ELISA not only contribute to the advancement of diagnostic tools but also serve as valuable educational resources for students [18].

Depamede et al. [19], successfully produced in-house polyclonal antibodies against rat meat extract using laying hens. In the present study, we describe the application of these antibodies in the development of a homemade sandwich ELISA for the detection of rat meat extract.

2. Materials and Methods

A. Biological Materials

The procedures in this study were adapted from Depamede et al. [19], unless specified otherwise. Rat meat was obtained from house rats, which were captured and processed in compliance with relevant regulations. The primary antibodies used in this study were produced in earlier research by Depamede et al. (19), where Hy-Line Brown laying hens were immunized with rat meat extract (RME), following a modified protocol based on Nurhaerani et al. [20]. The antibody production adhered to animal ethics guidelines under permit No: 361/UN18.F7/ETIK/2023. The secondary antibody employed was a commercially available Goat Anti-Rat IgG (H+L), conjugated with horseradish peroxidase (HRP), sourced from Elabscience, USA.

B. Sandwich ELISA

The sandwich ELISA was conducted using a 96-well high-binding ELISA microplate with 8-well strips and flat-bottom wells. Each well was coated with 100 µL of primary antibody (10 µg/mL chicken anti-rat IgY) diluted in phosphate-buffered

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Table 1
Outcomes of homemade sandwich ELISA analysis

Dilution Factors	Mean OD450 readings for the three RME treatment groups (n = 4)		
	Raw	70°C	100°C
1/10	1.3309 (P)	1.011 (P)	0.27517 (N)
1/1,000	0.7737 (P)	0.47207 (P)	0.24902 (N)
1/10,000	0.4503 (P)	0.3097 (N)	0.23967 (N)

P = Positive; N = Negative, determined using a cutoff value of 0.3213 (calculated as the mean plus, three standard deviations of the negative controls), as described by [21] and [22].

saline (PBS, pH 7.4) and incubated overnight at 4°C. Following incubation, wells were washed three times with 250 µL of PBST (PBS containing 0.05% Tween 20). Non-specific binding sites were blocked using 4% (w/v) skimmed milk in PBST for 60 minutes at 37°C. After another set of three washes, the wells were loaded with 50 µL of rat meat extract (REM) - either fresh or heat-treated at 70°C or 100°C for 15 minutes - diluted to various concentrations in PBS containing 1% bovine serum albumin (PBS-BSA). The plate was incubated at 37°C for 60 minutes and washed again three times with PBST. Subsequently, 50 µL of secondary antibody diluted in PBS-BSA was added to each well and incubated for 60 minutes at 37°C. After washing, 50 µL of TMB substrate was added to each well and incubated for 5–15 minutes in the dark. The enzymatic reaction was stopped by adding 50 µL of 1 M sulfuric acid to each well, and the absorbance was measured at 450 nm using an ELISA reader.

C. Data analysis

The ELISA data were analyzed using basic descriptive statistics, specifically the arithmetic mean. Variability among experimental groups was assessed using Microsoft Excel 365. Each data point represents the average of quadruplet measurements, and the entire experiment was independently repeated twice. Statistical significance was determined at a threshold of $P < 0.05$. The cut-off formula of the form "mean + 3 standard deviation of negative controls" was implied based on Lunn et al. [21] and Lardeux et al. [22].

3. Results and Discussion

This study aims to utilize polyclonal antibodies previously developed against rat meat extract (RME) [19] to establish a homemade sandwich ELISA capable of detecting the presence of RME in food products. This approach addresses the issue of meat adulteration, where rat meat, due to its lower cost compared to beef, chicken, or goat, is illicitly mixed into processed foods [23].

The antibody from Depamede et al. [19] was employed as the capture antibody in the assay. RME antigens were tested under three conditions, i.e. fresh, thermally treated at 70°C, and 100°C for 15 minutes. Each was diluted at 1:10, 1:1,000, and 1:10,000 using PBS with 1% BSA. ELISA results are shown in Table 1.

The developed ELISA demonstrated the ability to detect fresh RME at dilutions up to 1:10,000 (equivalent to 2.5 µg/mL). RME heated at 70°C was detectable up to a 1:1,000 dilution (25 µg/mL), while RME treated at 100°C was undetectable, likely due to heat-induced protein denaturation.

Compared to the assay developed by Chen et al. [14] which could detect rat protein at 0.01 µg/L in both raw and cooked samples, the sensitivity of the present ELISA is lower. A key methodological difference is that Chen et al. used thermally processed antigen (55°C for 60 minutes) as the immunogen, whereas this study used fresh RME.

Despite lower sensitivity, the assay is still effective for practical detection of rat meat in adulterated food, as economic adulteration is unlikely to involve dilutions beyond 1:1,000. ELISA's capability to detect RME heated at 70°C, a common processing temperature in products like sausages, supports its potential application in food safety monitoring. Additionally, it can serve as a useful tool for laboratory teaching and student training.

4. Conclusion

The findings of this study demonstrate that locally produced chicken anti-rat polyclonal antibodies are suitable for the development of a homemade sandwich ELISA for the detection of rat meat components. The assay successfully detected antigens from raw rat meat and thermally processed rat meat (heated at 70°C), with detection sensitivities reaching up to 1:10,000 dilution (2.5 µg/mL) and 1:1,000 dilution (25 µg/mL), respectively.

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