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Sensitivity of commercial rapid test kit to pork contamination in processed foods

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ABSTRACT

Guaranteeing halal products has now become a necessity, especially for food products. This is intended to ensure that the food produced is not contaminated with non-halal ingredients, including pork. Pork contamination in processed meat foods such as meatballs is still often found. Various tests can be done to detect the presence of pork in processed foods. One of them is a rapid test using the LFIA method. This test is widely used because it is more efficient, economical, and easy to prepare samples. A rapid pork contamination test kit (XEMA) has been circulating in Indonesia. In the research, the sensitivity of this rapid test kit was tested on processed meat foods with various concentrations of pork and variations in the main ingredients. The color test shows that the simulated samples of beef meatballs without added pork are dark greyish white, as are the simulated samples with concentrations of 1% and 10%. Meanwhile, samples with concentrations of 20% and 40% have a paler color. Meanwhile, there was no significant difference in the variation in pork concentration in meatballs with the main ingredients of chicken and fish. For smell and texture, there were no significant differences in the simulated samples, both the control and samples with varying concentrations. From testing, it is known that the test kit can detect the presence of pork up to a concentration of 10% in samples, with the main ingredients being beef, chicken, or fish. These results indicate that this rapid test kit can well detect pork contamination in processed food samples.

Keywords: Detection, Immunochromatography, LFIA, Pork

INTRODUCTION

The development of processed foods made from meat is taking place very rapidly. There are many types of products and price variations on the market (Ahda et al., 2022). This condition makes people need to increase awareness and vigilance regarding their food composition. One of the issues is pork contamination in processed meat foods on the market. This contamination can occur due to the manufacturer's intention or due to the use of equipment together with products made from pork (Ha et al., 2017)

Products with halal guarantees tend to increase. To provide comfort and certainty about the halalness of consumer products, the government has issued regulations regarding halal product guarantees, which business actors must fulfill to obtain halal certification (UU BPJPH.Pdf, n.d.). This certification will not only provide a sense of comfort, security, safety, and certainty about the availability of halal products for the community. However, it can also provide added value for business actors. The government is accelerating halal certification for consumer goods (Ahda et al., 2022).

Pork contamination in food can be in the form of meat (pork) and lard. Reported cases of pork contamination were found in meatballs (Effendi et al., 2020). This condition can occur for economic purposes or because of the use of meat grinding equipment together. Apart from that, it is also possible that producers cannot recognize the characteristics of pork, so they make mistakes when preparing raw materials. This condition is, of course, very detrimental to consumers (Murti et al., 2015).

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Proper identification is needed to determine the presence of pork contamination to guarantee the halalness of consumer products. Several methods have been reported to identify the presence of pork and lard in processed foods. Identification is carried out based on visual observation, fatty acid profiles (Rohman & Man, 2008), the presence of proteins and peptides (Konduru et al., 2021; Magdalena et al., 2021; Qin et al., 2021; Rölfing et al., 2021), and DNA (Balakrishna et al., 2019; Timakova et al., 2018; Yang et al., 2018). Each method has certain specificities. Real-time PCR is a method that is considered accurate because it can detect the presence of contamination very sensitively and is not influenced by heating conditions during material processing (Effendi et al., 2020; Kissenkötter et al., 2020). However, it requires complex steps and resources. So, currently, we need a test method that is fast, accurate, efficient, and economical. One thing that is starting to be developed is a rapid testing tool. In Indonesia, immunochromatography-based and fatty acid content-based test kits have been circulating for pork contamination (Al-Kahtani et al., 2014; Azir et al., 2017; Azizan et al., 2021). However, it is necessary to test the detection capabilities of identification tools circulating in Indonesia as a reference for testing the halalness of processed meat-based food products.

RESEARCH METHOD

The research was conducted at the Chemistry Laboratory, Faculty of Science and Technology, UIN Sulthan Thaha Saifuddin Jambi.

Materials

Pork, beef, chicken, and fish are obtained from local markets. The equipment required includes micropipettes, 1 ml tips, microtubes, 70% alcohol, alcohol swabs, rapid detection kits (XEMA), and a digital pH meter.

Methods

1. Making meatball simulations with various concentrations of pork
   To study the sensitivity of commercial detection kits, simulated samples were prepared by mixing pork into beef, chicken, and fish meatball mixtures with a concentration range of 1, 10, 20, and 40 (%w/w).
2. Sample Extraction
   100 grams of each sample and control were chopped and ground. 10 g of the ground sample was added to distilled water, homogenized, and then centrifuged at a speed of 3000 x g for 30 minutes. Take the supernatant, then aliquot 1 ml each for testing. Swab samples of the cutting tools used to cut and chop samples were also tested.
3. Organoleptic Test
   Organoleptic tests were carried out with color, aroma, and texture parameters using a single panelist.
4. pH Test
   pH testing was carried out on all simulation samples.
5. Sensitivity Test
   Testing procedures will be carried out based on the detection kit manual.

RESULT AND DISCUSSION

The test stage begins with collecting fresh raw beef, chicken, fish, and pork samples from local traders. Making meatball simulation samples is done by mixing ground pork with meatball dough, which has the main ingredients of beef, chicken, and fish in predetermined ratios, namely 1%, 10%, 20%, and 40%. Then, the dough is rolled into balls and boiled, like meatballs. Meatballs without added pork were prepared as a control.

Organoleptic Test

The test uses parameters like color, smell, and texture. For smell and texture, there were no significant differences in the simulated samples, both the control and samples with varying

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concentrations. In making the simulation samples, several kitchen spices commonly used to make meatballs were added to study their effect on KIT detection ability.

Table 1. Organoleptic Test Results with color, texture, and aroma parameters

Sample Code	Color	Texture	Aroma
S1	Greyish white	Chewy	Typical meatballs
S10	Greyish white	Chewy	Typical meatballs
S20	Pale Greyish white	Chewy	Typical meatballs
S40	Pale Greyish white	Chewy	Typical meatballs
A1	Pale Greyish white	Chewy	Typical meatballs
A10	Pale Greyish white	Chewy	Typical meatballs
A20	Pale Greyish white	Chewy	Typical meatballs
A40	Pale Greyish white	Chewy	Typical meatballs
I1	White	Chewy	Typical fish balls
I10	White	Chewy	Typical fish balls
I20	White	Chewy	Typical fish balls
I40	White	Chewy	Typical fish balls

Description: S1: beef meatballs with 1% pork concentration; S10: beef meatballs with 10% pork concentration; S20: beef meatballs with 1% pork concentration; S40: beef meatballs with 40% pork concentration; A1: chicken meatballs with 1% pork concentration; A10%: chicken meatballs with 20% pork concentration; A40%: chicken meatballs with 40% pork concentration; I1%: fish balls with 1% pork concentration; I10%: fish balls with 10% pork concentration; I20%: fish balls with 20% pork concentration; I40%: fish balls with 40% pork concentration.

The color test shows that the simulated samples of beef meatballs without added pork are dark greyish white, as are the simulated samples with concentrations of 1% and 10%. Meanwhile, samples with concentrations of 20% and 40% have a paler color. Meanwhile, in meatballs with the main ingredients of chicken and fish, there was no significant difference in the variation in pork concentration (Table 1).

The color of meat is influenced by the concentration of myoglobin and its redox stability. Myoglobin itself is a water-soluble hemoprotein and has iron as a prosthetic group. This iron's presence determines myoglobin's redox state through its valence state and associated ligands with free coordination. In fresh meat, myoglobin can be in one of three states of dynamic equilibrium: deoxymyoglobin, oxymyoglobin, or metmyoglobin. Myoglobin can chemically interact with various components in meat products, influencing their redox state. Structural changes in myoglobin caused by processing conditions will affect thermal stability and consequently change the color of meat products (Zvereva et al., 2020). The myoglobin content is higher in beef and lower in poultry, while lamb and pork have moderate amounts. The age of the animal will also impact the myoglobin content of the muscle, as older animals have more myoglobin and darker meat (Zvereva et al., 2020)

pH Test

Apart from organoleptic aspects, pH is one of the basic parameters that can be used to identify types of meat. Each type of meat has a specific pH range, especially in fresh and unprocessed conditions, whereas, in meat that has undergone processing, there is a slight shift in the pH value when compared to fresh conditions. This research identified the pH value for each test sample with varying concentrations of pork and the main ingredients.

From pH measurements, it is known that the pH of beef meatballs with the addition of 1% pork is 5.57 and increases with increasing pork concentration. At a pork concentration of 10%, a pH value of 5.58 was obtained, and with the addition of 20% pork, a value of 5.59 was obtained, while at the addition of 40% and 60%, the pH values were obtained at 6.01 and 6.03. There was no significant change in the pH value for the main ingredient, chicken, due to the addition of pork. At pork meat concentrations of 1%, 10%, and 20%, a pH value of 5.92 was obtained, while when adding pork at a concentration of 40%, a pH value of 5.90 was obtained. In simulated samples with fish as the main

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ingredient, pH tended to decrease along with increasing pork concentration. This can be seen in the successive decrease in pH values, namely 6.36, 6.35, 6.34, and 6.32, for variations in pork concentration of 1%, 10%, 20%, and 40% (Figure 1). The difference in pH for each main ingredient is influenced by the amount of protein, the type of amino acids that make up it, and the fatty acid components contained in the food ingredient.

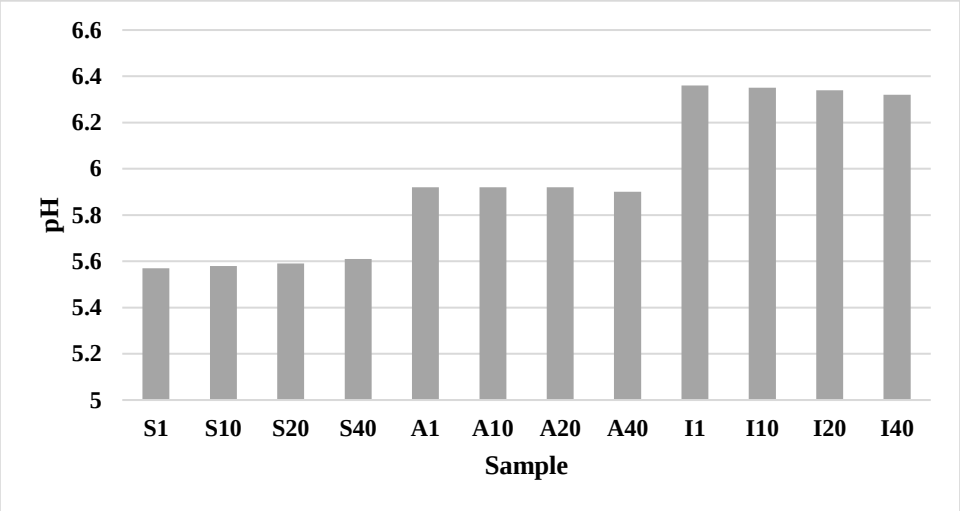

Figure 1. pH Values in simulated sample variation. S1: beef meatballs with 1% pork concentration; S10: beef meatballs with 10% pork concentration; S20: beef meatballs with 1% pork concentration; S40: beef meatballs with 40% pork concentration; A1: chicken meatballs with 1% pork concentration; A10%: chicken meatballs with 20% pork concentration; A40%: chicken meatballs with 40% pork concentration; I1%: fish balls with 1% pork concentration; I10%: fish balls with 10% pork concentration; I20%: fish balls with 20% pork concentration; I40%: fish balls with 40% pork concentration.

Sensitivity Test

Simulation samples were prepared according to the kit manual. Tests were carried out for control and simulation samples with concentration variations of 1%, 10%, 20%, and 40% for ready-to-consume meatball samples and cutting tool swab samples during simulation sample preparation. Each solid sample was weighed with the same weight, namely 5 g. Testing is carried out by dipping the test part of the KIT into the sample extract for 15 seconds to ensure the sample flows through the capillary and then waiting for 30 seconds before reading. The appearance of a red color in the test area indicates a positive result of pork contamination.

From testing, it was discovered that the kit showed positive results in the form of red color in the test area for samples S10, S20, and S40, which were simulated samples using broom meat as raw material with a pork content of 10%, 20%, and 40%. Meanwhile, no positive results were found in the simulation samples with a concentration of 1% and the cutting tool swab samples. At a concentration of 10%, the red color on the test line is still faintly visible. From these observations, it is known that this tool can identify the presence of pork contamination up to a concentration of 10% for processed meat samples.

Immunology testing is one method that has the potential to be used to identify pork contamination in food ingredients. A test format that is rapidly being used and is developing is immunochromatographic analysis, or lateral flow immunoassay (LFIA) (Hendrickson et al., 2021). This test uses a test strip composed of a membrane with many pores through which the immobilized antibody reagent can pass. Contact between the liquid sample and the test strip initiates the movement of the reagent across the membrane, followed by the formation of an antibody complex that can be detected in certain areas on the strip. The test results are signals that can be seen through certain systems(Murti et al., 2015; Zvereva et al., 2020).

Table 2. Sensitivity test result

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Sample Code	Pork (%)	Test result
S1	1	-
S10	10	+
S20	20	+
S40	40	+
A1	1	-
A10	10	+
A20	20	+
A40	40	+
I1	1	-
I10	10	+
I20	20	+
I40	40	+
E	Cutter swab	-

Description: S1: beef meatballs with 1% pork concentration; S10: beef meatballs with 10% pork concentration; S20: beef meatballs with 1% pork concentration; S40: beef meatballs with 40% pork concentration; A1: chicken meatballs with 1% pork concentration; A10%: chicken meatballs with 20% pork concentration; A40%: chicken meatballs with 40% pork concentration; I1%: fish balls with 1% pork concentration; I10%: fish balls with 10% pork concentration; I20%: fish balls with 20% pork concentration; I40%: fish balls with 40% pork concentration.

In this commercial kit, the test basis used is the presence of specific antigen proteins that some organisms possess and still remain in meat components (Kuswandi et al., 2017). In this kit, porcine ultra-heat-resistant mucin is used as the antigen. To determine the level of sensitivity of the tool, the test was carried out on simulated samples mixed with beef and other animal protein ingredients widely consumed by the public, especially those widely processed as meatballs. The samples consisted of chicken and fish. Test samples for chicken and fish were made with the same composition as beef, namely 1%, 10%, 20%, and 40%, and made by adding kitchen spices, which are usually used in making meatballs, and heating until the texture turns solid and cooked.

The test results using the kit showed that the simulated sample with a mixture of chicken gave faint positive results at a pork meat concentration of 10% and was quite clear at concentrations of 20% and 40%. The same results were also shown in testing samples with fish ingredients. At a concentration of 1%, the test equipment showed negative results. These results were seen in the basic samples of chicken and fish (Table 2). These results indicate that the rapid detection kit circulating in Indonesia accurately detects the presence of pork contamination.

CONCLUSION

A rapid commercial kit for pork detection has good sensitivity and could be an alternative tool to first-step testing for pork contamination.

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