TASP 2016

Jointly organized by

Volume II

PROCEEDINGS

1st International Conference on

Tropical Animal Science and Production (TASP 2016)

"Integrated Approach in Advanced Animal Science and Innovation Technology"

July 26-29, 2016
Ambassador Hotel, Bangkok, Thailand

TASP 2016

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Volume II

Proceedings

1st International Conference on
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"Integrated Approach in Advanced Animal Science and Innovation Technology"

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Jointly organized by:

Suranaree University of Technology

Rajamangala University of Technology Isan

Nakhon Ratchasima Rajabhat University

Thailand Research Fund

Welcome Letter from the Rector of SUT

Dear Participants,

I would like to welcome all of you who have come to Bangkok, Thailand, from all over the world. We are proud and honored to host the 1st International Conference on Tropical Animal Science and Production (TASP 2016). This year, Suranaree University of Technology is celebrating its 26th Anniversary. Over the past 26 years, we are proud to be consistently ranked as one of Thailand's leading universities, with a strong reputation for research excellence and impact. This Conference is certainly a wonderful addition to our calendar of 26th anniversary celebration activities.

The theme of this conference "Integrated Approach in Advanced Animal Science and Innovation Technology" is appropriate in view of the orientation of technological and institutional changes in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations.

If you take a look at the conference program, you will agree that this conference is going to be busy and productive. There is a lot to offer for a four-day event. I would like to take this opportunity to thank the Conference Organizing Committee for their diligent work. I would also like to thank participants, especially those of you coming from abroad, for joining us and sharing your valuable experience and ideas. I hope this conference provides you with a forum to exchange scientific ideas, inspire new research, and new contacts for closer co-operation, so that we can, together, envision the future of a promising development of the animal science and production in the Tropic and all over the world.

Last but not least, I sincere thank our co-hosts, namely, Rajamangala University of Technology Isan (RMUTI), Nakhon Ratchasima Rajabhat University (NRRU) and The Thailand Research Fund (TRF), and our sponsors for their immense support in making this conference successful. In closing, I wish you many splendid ideas, new partnerships and the best of success, and I wish our visitors from abroad will have a very pleasant stay in the beautiful city of Bangkok.

Sincerely,

P. Suebka.

Prof. Dr. Prasart Suebka
Rector

Message from the Chairman of TASP 2016

Dear Participants,

On behalf of the Organizing Committee, I would like to welcome you to The 1st International Conference on Tropical Animal Science and Production 2016 (TASP 2016) in Bangkok, Thailand, from 26th to 29th July 2016. The theme of this conference is "Integrated Approach in Advanced Animal Science and Innovation Technology" to appropriate in view of the orientation of technological and institutional changes in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. The purpose of the conference is to provide an opportunity for animal scientists, animal agriculture-crop scientists and those from related disciplines to discuss and debate research issues relating to aquaculture, animal behavior and welfare, animal genetics and breeding, animal physiology and reproduction, animal biotechnology, epigenetics, feed science and technology, livestock farming system, livestock management, meat science, non-ruminant nutrition, nutrigenomics, pasture, pet, ruminant nutrition, veterinary and others.

The 1st TASP 2016 is organized by 4 institutes; Suranaree University of Technology (SUT), Nakhon Ratchasima Rajabhat University (NRRU), Rajamangala University of Technology Isan (RMUTI) and The Thailand Research Fund (TRF).

I sincerely hope that the papers presented in the 1st TASP 2016 would cover all major areas animal science and production and yield meaningful information leading to further strengthening of animal science and production in the tropical areas.

I would like to thank all authors for submitting their works and all members of Local and International Organizing Committee, reviewers, all co-host institutions, sponsors without the support of whom a conference of this scale would not have possible, and all individuals who have contributed greatly to the success of this conference.

Sincerely,

Assoc. Prof. Dr. Pramote Paengkoum
Chairman,
Organizing Committee TASP 2016

Message from the Chairman of Academic Committee

Dear Participants,

On behalf of Academic Committee, it is my great pleasure to welcome you to the 1st International Conference on Tropical Animal Science and Production (TASP 2016) in Bangkok, Thailand.

The conference has been designed to provide an opportunity for animal scientists, researchers and research scholars to exchange and to share their experiences, and to promote awareness of the ongoing research achievements. It also provides the premier interdisciplinary forum for animal scientists to present their latest research results, ideas, developments, and applications in all areas of Animal Science and Production. The conference will feature a program of keynote and plenary speakers, invited speaker and oral sessions, posters, and workshops.

I would like to express my sincere thanks for all keynote speaker, plenary speakers, invited speakers and all participants for their contribution to make this scientific meeting a great success and fruitfulness. It is our great honour to have Prof. Dr. Charan Chantalakhana from Thailand, Prof. Dr. Metha Wanapat from Thailand, Prof. Dr. Liang Chou Hsia from Taiwan, Assoc. Prof. Dr. Jowaman Khajarern from Thailand, Prof. Dr. Junichi Takahashi from Japan, Assoc. Prof. Dr. Pongchan Na-Lampang from Thailand, Dr. Pascal Mermillod from France, Dr. John Moran from Australia, Prof. Dr. Yang Sheng Lin from China, Prof. Dr. Mongkol Techakumphu from Thailand, Prof. Dr. Thomas J. Schonenwille from the Netherlands and Prof. Dr. Nguyen Van Thu from Vietnam, who are the esteemed speakers and specialists on their own fields to joint us in this conference. I also deepy appreciate the scientific committee, reviewer and editorial boards for their great contribution to make this conference efficacious.

I hope that you will enjoy the conference and your interaction with your colleagues from many different countries. Also, I wish that this conference will stimulate a creative exchange of ideas and opportunity for networking, collaboration, sharing of technical information and the building of trust relationship internationally. Last but not least, I am confident that you will enjoy your visit in Bangkok and find a harmonious blend of culture, cuisine, arts, and nice sightseeing along excellent shopping opportunities.

Yours sincerely,

Sutisa Khempaka

Asst. Prof. Dr. Sutisa Khempaka
Chairman of Academic Committee

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Contents

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Species specific polymerase chain reaction (PCR) assay for identification of pig (*Sus domesticus*) skin in "Rambak" crackers

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Abstract

"Rambak" is the animal skin crackers product which one of the popular food products derived from the animal by-products in Indonesia. For the religion reasoning the presence of pig derivatives in any food products is prohibited for Moslem community. For this reason, the analytical methods offering accurate and sensitive results are highly needed in order to assure the halal authenticity of food. The objectives of this research is to developed the pig species specific primer for identification of specific pig deoxyribonucleic acid (DNA) in "Rambak" cracker using polymerase chain reaction (PCR) analytical methods. This research used were four kinds of animal skin crackers in individual or mixture samples. Skin mixture were prepared and divided into nine formulas as follows 1) 100% pig; 2) pig 90%: cattle 10%; 3) pig 75%: cattle 25%; 4) pig 50%: cattle 50%; 5) pig 25%: cattle 75%; 6) pig 10%: cattle 90%; 7) pig 1%: cattle 99%; 8) pig 0.1%: cattle 99,9%; and 9) 100% cattle. The same formula also prepared in mixture between pig skin with buffalo and goat skin. DNA were isolated from the cracker products and applied in PCR using perimer specific. The optimized PCR assay was subsequently validated for its specificity with deoxyribonucleic acid (DNA) extracted from cattle, buffalo, goat and pig in individual or mixture samples. Results showed the primers designed generated specific fragment of 510 bp length for pig cytochrome b DNA. The specificity of the primers was tested on four animal species including pig, cattle, buffalo and goat species. Analysis of experimental mixture meat demonstrated that 0.1% of pig tissues could be detected using specific primer. The specificity of pig-specific PCR provides a valuable tool for identification of pig skin and to avoid its fraudulent substitution and adulteration.

Keywords: pig species, identification, primer specific, cytochrome b gen

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Introduction

"Krupuk Rambak" or Rambak cracker, is one of the traditional popular food in Indonesia, made from thick skin of domestic animal. This food is traditionally hand made with traditional process. One of the interesting issues in food industry is the authenticity of food products. The quality assurance of food product can involve the alteration of the true labeling of food ingredients,

in which the high value of raw materials are substituted by cheaper materials with similar biochemical properties. Cattle or buffalo hide are expensive and can be altered with cheaper skin such as pig skin.

The presence of pig derivatives such as pig skin, lard, pork and porcine gelatin in any food products is a serious matter for moslem community (Mursyidi, 2013), and also for Judaism and Hinduism because some forbid their followers to consume any foods containing porcine and its derivatives (Regenstein et al., 2003). Therefore, it is necessary to evaluate the presence of these pig derivatives in any products using some instrumental techniques. Some previous techniques used are differential scanning calorimetry (Nurrulhidayah et al., 2015; Marikkar et al., 2012), Fourier transform infrared (FTIR) spectroscopy (Rohman et al., 2011), gas chromatography-mass spectrometry (Nizar et al., 2013), high pressure liquid chromatography (Saeed et al., 1989; Marikkar et al., 2005), electronic nose (Nurjuliana et al., 2011; Mansor et al., 2011), and proton nuclear magnetic resonance (Fang et al., 2013). Some of these methods are too laborious and time consuming, consequently, an analytical technique offering rapid and reliable must be used. One of the promising methods suitable for routine analysis is polymerase chain reaction- restriction fragment lenght polymorphism (PCR-RFLP).

In the previous research we already developed the new technique based on the PCR-RFLP technique for meat products determination using cytochrome b gen using universal primer and resulted 360 bp DNA fragment. The determination was continued with restriction fragment length polymorphism using BseD I restriction enzyme and able to cleave amplicon of cytochrome b gen into 221 bp and 138 bp (Erwanto et al., 2012). This technique has been applied in detection of pig derivative contamination in commercial meatball of Indonesia local market and showed positive pig meat contamination of nine from thirty-nine samples (Erwanto et al., 2014). PCR-RFLP technique has been established to determine of meat species contamination but still time consuming and need two stage methods, first the amplification of gen target with universal primer and the second, cleave PCR products using restriction enzyme. Therefore, in this study we developed the species specific primer for distinguish of pig derivative particle based on cytochrome b gen in food derivative products from animal skin.

Materials and Methods

Sample preparation and DNA extraction

Individual skin crackers samples of pig, buffalo, cattle and goat were obtained from local market in Yogyakarta, Indonesia. Samples was prepared in laboratory with separate equipment to prevent cross contamination. DNA was extracted and purified from skin cracker samples using Sambrook et al. (1989) methods with slight modification. Approximately 30 mg of skins were blended using a commercial blender and placed in a 1.5 mL microcentrifuge tube. A-500 µL of Tris-EDTA-NaCl (TEN) buffer, 10 µL Proteinase K and 50 µL 10% Sodium Dodecyl Sulfate (SDS) were added and mixed by vortexing and was incubated at 42°C in a water bath overnight. After overnight incubation, 50 µL5 M NaCl, 400 µL phenol and 400 µL chloroform-isoamyl-alcohol was added to the mixture and then incubated at 37°C for 1 h and centrifuged at 3,000 rpm for 5 min. The supernatant was taken and put in a new microcentrifuge tube and mixed by vortexing for 30 s, then 50 µL 5 M NaCl and 1 mL absolute ethanol were added, mixed vigorously and incubated at –20°C for 1 h. The DNA was precipitated from the mixture after centrifuging at 8,000 rpm for 5 min, 4°C and the supernatant discarded. The 70% ethanol was gently poured off then resuspended DNA for a second precipitation. The solution was then placed at room temperature overnight. The resulting pellet was added with 50 mL Tris-EDTA (TE) buffer and stored at –20°C

for further analysis. Skin cracker samples were prepared by mixing of pig in buffalo, pig in cattle, and pig in goat at a final concentration of pig at 0; 0.1; 1; 10; 25; 50; 75; 90 and 100%.

Design of pig specific primer

The nucleotide sequences of mitochondrial Cyt b genes from sus scrofa (pig) stored in Genbank (www. ncbi. nig. gov) were taken and aligned using Primer3 website (www.primer3.ut.ee/). The set of primers used for amplification consisted of Cyt b-FW and Cyt b-REV oligonucleotides as follows:

Cyt b FW 5'-CCC AGC CCC CTC AAA CAT CTC A-3',
Cyt b REV 5'-ATG TAC GGC TGC GAG GGC GGT-3'.

Amplification of the *Cyt b* gene was performed in a final volume of 25 µL containing 100 ng of extracted DNA, 20µL DreamTaq Green PCR master Mix (Thermo Fisher Scientific) and 20 µmol of each primer. Amplification was performed with a thermal cycler according to the following PCR step-cycle program: Pre-denaturation at 94°C for 2 min to completely denature the DNA template, followed by 40 cycles of denaturation at 95°C for 36 s, annealing at 53°C for 73 s. and extension at 72°C for 84 s. Final extension at 72°C for 3 min followed the final cycle for complete synthesis of elongated DNA molecules. Six microliters of PCR products were mixed with 1 µL DNA Loading Dye (Thermo Fisher Scientific), then were electrophoresed at constant voltage (100 V) on 2% agarose gel (TopVision, Thermo Fisher Scientific) for about an hour in 1×TBE buffer, pH 8.0 and stained by ethidium bromide. A-100 bp DNA ladder (GeneRuller, Thermo Fisher Scientific) was used as size reference.

Results and Discussion

Total genom DNA form the various animal skin species was isolated using Sambrook et al. (1989) method with slight modification. During processing proteins are denaturalised due to the time and temperatures of process and DNA should be employed for species identification purposes. Therefore the susceptibility of the determination of species in food products depend on the susceptibility in DNA extraction, without the extracted DNA the measurement of the detection could not be continued.

DNA genom of four species could be successfully isolated (data not shown) although there were some smear in goat skin sample. The quality of DNA and the level content of pig in the crackers mix samples is important data for verifying the sensitivity of the method.

To identify pig skin in a large number of both processed and unprocessed skin crackers, we carried out specific pig PCR amplification. With this fragment, pig has been detected in mixtures treated with cattle, buffalo and goat skin crackers. Some commercial products were analyzed which normally have cattle, buffalo or pig in their crackers product including in fresh skin crackers or various processed skin crackers. Using the specific PCR amplification, we detected pig in various concentration. Pig was detected until 0.1% contamination in mixture with cattle (Figure 1), buffalo (Figure 2) or goat (Figure 3). In our some previous research showed the PCR-RFLP able to detect pig DNA in heated meat mixtures at levels less than 1% based on fragment of the cytochrome b DNA (Erwanto et al., 2012) and Matsunaga et al. (1999) detected 250 pg of beef DNA, in DNA mixtures. In raw pork and beef mixtures, Calvo et al. (2001) detected up to 0.01% beef in pork.

The choice of the technique to be applied is based on the results of sequence alignments. Specific PCR seems to be best as a 'routine' test, because it is easy, rapid and allows the discrimination of several species at the same time (multiplex PCR), as well as the analysis of mixed

matrices. It is considered to be a robust method, mainly in comparison with other methods based on the analysis of single nucleotide polymorphisms (Bottero and Dalmaso, 2011).

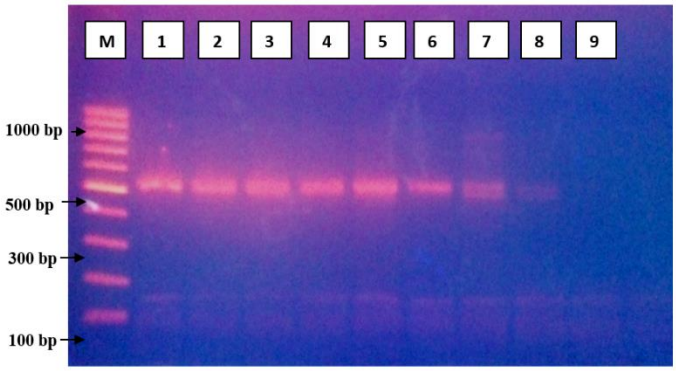

Figure 1. PCR products of cyt b specific gene from different skin product separated by 2% high-resolution agarose gel electrophoresis. M: Marker; 1: 100% pig skin; 2: pig 90%: buffalo 10%; 3: pig 75%: buffalo 25%; 4: pig 50%: 50% buffalo; 5: pig 25%: 75% skin; 6: pig 10%: buffalo 90%; 7: pig 1%: 99% buffalo; and 8: pig 0.1% buffalo 99.9%; 9: 100% buffalo skin.

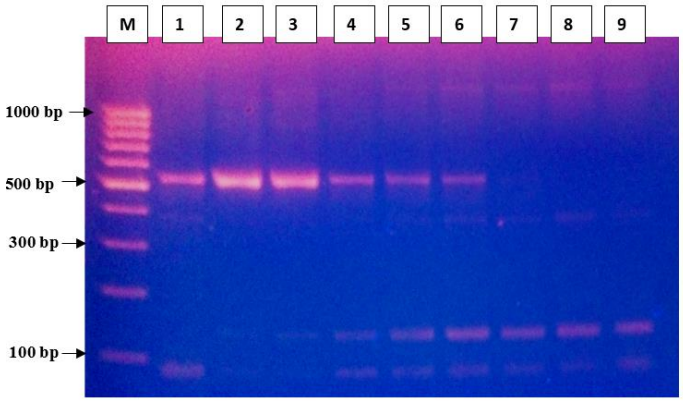

Figure 2. PCR products of cyt b specific gene from different skin product separated by 2% high-resolution agarose gel electrophoresis. M: Marker; 1: 100% pig skin; 2: pig 90%: cattle 10%; 3: pig 75%: cattle 25%; 4: pig 50%: 50% cattle; 5: pig 25%: 75% cattle; 6: pig 10%: cattle 90%; 7: pig 1%: 99% cattle; and 8: pig 0.1% cattle 99.9%; 9: 100% cattle skin.

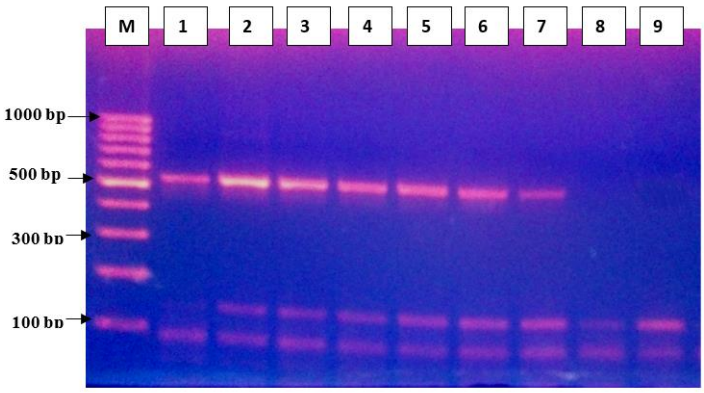

Figure 3. PCR products of cyt b specific gene from different skin product separated by 2% high-resolution agarose gel electrophoresis. M: Marker; 1: 100% pig skin; 2: pig 90%: goat 10%; 3: pig

75%: goat 25%; 4: pig 50%: 50% goat; 5: pig 25%: 75% goat; 6: pig 10%: goat skin 90%; 7: pig skin 1%: 99% goat; and 8: pig skin 0.1% goat skin 99.9; 9: 100% cattle skin.

Conclusion

Specific PCR amplification of this fragment is a powerful technique for the identification of pig contamination, due to its simplicity, specificity, and sensitivity (with 35 amplification cycles we can detect 0.1 % pig skin). However, further research would be needed in order to develop a quantitative method since this conventional PCR analysis is only qualitative.

Acknowledgment

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