EXPERIENCE REPORT
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# Microscopic and molecular identification of foreign matter in food: detection of fraudulent practices Identification of foreign matter: food fraud

## Identificação microscópica e molecular de matérias estranhas em alimentos: detecção de práticas fraudulentas

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## ABSTRACT

**Introduction:** The most frequent demands on microscopic food analysis are allegations of consumers finding macroscopic foreign matter or suspecting the presence of undeclared ingredients on products labels. The byproducts and foreign matters detection are fundamental practice for indirectly verifying the conditions of food production. **Objective:** This study reports the processes of microscopic and molecular identification (PCR) of a foreign matter found in a meat pie after a consumer complaint, occurred in the city of Itapira, state of São Paulo, Brazil. **Method:** Two distinct procedures were used to identify foreign matter: macroscopic examination, following FDA standards, and polymerase chain reaction (PCR) technique to identify DNA extracted from foreign materials. **Results:** The macroscopic analysis identified animal taste buds composing the pie fillings, and the PCR test confirmed that they were of bovine origin. **Conclusions:** Macroscopic analysis and the PCR test allowed the identification of the type of foreign matters and confirmed its bovine origin, what was enough to characterize it as a fraud by the improper use of inferior tissues in the preparation of ready-to-eat pastry.

**KEYWORDS:** Microscopic Identification; Molecular Identification; Foreign Matter in Food; Fraudulent Practices; Food Control

## RESUMO

**Introdução:** Uma das mais frequentes demandas de análise microscópica de alimentos são denúncias de consumidores que encontram matéria estranha macroscópica ou suspeitam da presença de ingredientes não declarados no rótulo do produto. A detecção de subprodutos e matérias estranhas é uma prática fundamental para verificar indiretamente a condição de produção de alimentos. **Objetivo:** Este estudo relata o processo de identificação microscópica e molecular (PCR) de uma matéria estranha encontrada em um pastel de carne após queixa de um consumidor no município de Itapira, estado de SP, Brasil. **Método:** Dois procedimentos distintos foram empregados para a identificação da matéria estranha: exame macroscópico seguindo padrões estabelecidos pelo FDA e técnica de reação em cadeia da polimerase (PCR) para identificação do DNA extraído da matéria estranha. **Resultados:** A análise macroscópica identificou a matéria estranha como sendo papilas gustativas de origem animal, e o teste da PCR confirmou que as mesmas eram de origem bovina. **Conclusões:** A análise macroscópica e o teste da PCR permitiram a identificação do tipo de matéria estranha e confirmação de sua origem bovina, caracterizando a fraude pelo uso indevido de tecidos inferiores na preparação de pastéis prontos para consumo.

**PALAVRAS-CHAVE:** Identificação Microscópica; Identificação Molecular; Matérias Estranhas em Alimentos; Práticas Fraudulentas; Controle de Alimentos

## INTRODUCTION

The Division of Health-Related Products and the Technical Group of Food (Brazilian State Health Secretariat) carry out health surveillance to promote and protect the population's health. These organizations are capable of eliminating or preventing risks arising from food. To this end, these organizations are responsible for performing scheduled monitoring of the sanitary quality of food products and establishments, and for responding to consumer complaints of adverse and dubious cases of food quality and safety[1].

In Brazil, the Ministry of Health through the Resolution n° 14 of March 28, 2014[2], defined undesirable parts or impurities as parts of plants or animals, such as peels, peduncles, petioles, cartilage, aponeurosis, bones, animal feathers and hair and carbonized particles of the food arising from or not removed by processing. Moreover, according to the Brazilian Ministry of Agriculture, the raw material to be used for the preparation of minced meat must be free of inferior tissues such as bones, cartilages, partial fat, aponeuroses, tendons, clots, and lymphatic nodes (Normative Instruction n° 83, of November 21, 2003)[3]. Subsequently, Decree n° 9.013, of March 29, 2017 – the Regulation on Sanitary and Industrial Inspection Service on Animal Origin Products (RIISPOA) – through Article 280[4] establishes that meat and offal used in the preparation of meat products must be free of fat, aponeuroses, lymph nodes, glands, gall bladder, pericardial sac, papillae, among others. Thus, fragments of taste buds are considered undesirable parts because they are defined as inferior tissues that are not permitted as ingredients in the manufacture of meat products, and the use of poor quality raw material or the intentional use of improper parts constitutes food fraud[5], and can be implicated in economic damages[6] and public health risk.

The study of foreign matter in foods, including dirt, decomposed material and miscellaneous materials is recommended by the Association of Official Analytical Chemists (AOAC) International with the assertion that these contaminants may be present due to inappropriate conditions or practices of production, storage, or distribution. One of the most frequent demands of microscopic food analyses comes from consumers who recognize macroscopic foreign matter in food or suspect the presence of ingredients that have not been declared on the labels. Moreover, foreign matter found in food is closely related to the rigor of compliance with good handling practices throughout the food production chain[7]. The detection of by-products and extraneous components is a fundamental practice conducted by the Health Surveillance to indirectly verify hygienic food producing conditions to reduce occurrence of impurities in the final product[8].

Thus, this experience report presents the results of an ordered and integrated microscopic analysis in addition to the identification of the animal species through a molecular analysis to validate consumer complaints of foreign matter present in food.

## METHOD

### Sample collection

The samples refer to a consumer complaint made at the Sanitary Surveillance of Itapira/SP (VISA) – referring to the presence of rigid fragments, foreign to the natural contents of pasty meat and suspected fragments of pork fat. A formal Surveillance complaint was registered on 13th/01/2016, being the inspection and the sample collection protocol executed in the same day. Samples were immediately forwarded to Adolfo Lutz Institute (IAL) – Campinas III Regional Center of Analysis Laboratory; simultaneously, the Sanitary Surveillance Technicians from Itapira/SP inspected the establishment responsible of commercializing the products, being the collection of new samples done, for inspection purposes, complementing the orientation analysis.

### Microscopic analysis

Samples for orientation analysis and inspection purposes were evaluated with respect to composition, quantity, apprehension data and other characteristics, such as storage conditions and inviolable packaging. Subsequently, investigations of macroscopic foreign matter were carried out, following standards laid down by the Food and Drug Administration[9] and the Association of Official Analytical Chemists[10].

The grey-colored foreign matter sample (Figure 1) was isolated in Petri dishes and identified using a stereoscopic microscope (magnification, 30x; Figure 2). Its microscopic characteristics were compared to existing standards in the Bank of Microscopic Patterns of Foreign Matters at the Morphology and Microscopy Department of the Central Laboratory of São Paulo/SP.

### Molecular analysis

The papillary fragments isolated from the samples were subjected to pre-treatment with trypsin-versene (ATV) for cell

**Figure 1.** Macro-analytical examination of the foreign matter sample, showing grey fragments suspected to be papilla of animal origin.

digestion and subsequent extraction of genetic material using a commercial QIAamp DNA Mini Kit-Qiagen®, USA kit. DNA from a bovine tongue pattern was also extracted for comparison.

Polymerase chain reaction (PCR) was performed for the extracted DNA, using primers for the cytochrome b (cytb) gene of *Bos taurus* and *Bos indicus*[11] and the feline glyceraldehyde-3-phosphate gene GAPDH[12], described in Table.

Each amplification reaction was performed in a 25.00 µL reaction volume containing 2.50 µL of sample DNA, 12.50 µL of Go taq Green Master Mix 2x (Promega, USA), 7.50 µL of ultrapure water, 1.25 µL of sense primer, and 1.25 µL of antisense primer at a concentration of 10.00 pmol/µL. For GAPDH, the following thermocycler program was used: initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94ºC for 20 sec, annealing at 55ºC for 30 sec and extension at 72ºC for 30 sec; and final extension of 72°C for 7 min. For Cytb, the following thermocycler program was used: initial denaturation at 95°C per min; 39 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec; and final extension at 72°C for 10 min.

The product was purified directly from the amplified single band obtained on an agarose gel by using ExoSap (Ge Healthcare) as per to manufacturer's instructions. The purified products were submitted to bidirectional sequencing, using Big Dye™ v 3.1 and the ABI-3500® automated sequencer (Applied Biosystems, Foster City, CA).

The chromatograms obtained for each DNA strand sequences of each sample were subjected to Phred application online (http://asparagin.cenargen.embrapa.br/phph/) to evaluate the quality of the same ones. Only positions with scores higher than 20 (less than 1% of error probability) were used and the chromatograms were also manually checked with the program BioEdit version 7.2.5 and submitted to BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

## RESULTS

### Microscopic analysis

A thorough microscopic examination revealed the presence of taste bud fragments suggestive of animal species (Figure 2). According to the current legislation, the presence of papillae, inferior tissue, is an irregularity.

Because the size of the papillae found in the samples was small compared to the bovine tongue pattern, there was a possibility of the papillae being of feline origin. Thus, the taste buds were sent to the Laboratory of Molecular Biology (Labmas), of the Department of Preventive Veterinary Medicine and Animal Health (VPS), of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (FMVZ/USP), in order to identify the species by using PCR.

**Table.** Primers for gene amplification.

| Primer | Gene | Sequences | Fragment size | References |
|---|---|---|---|---|
| GAPDH fel S | GAPDH | 5' GCCGTGGAATTTGCCGT 3' | 164 bp | Leutenegger CM, 1999[11] |
| GAPDH fel AS | | 5' GCCATCAATGACCCCTTCAT 3' | | |
| BoV1 S | Cytb | 5'GGCTTATATTACGGGTCTTACACT 3' | 279 bp | Bottero et al., 2002[12] |
| BoV2 AS | | 5'GGCAATTGCTATGATGATAAATGGA 3' | | |

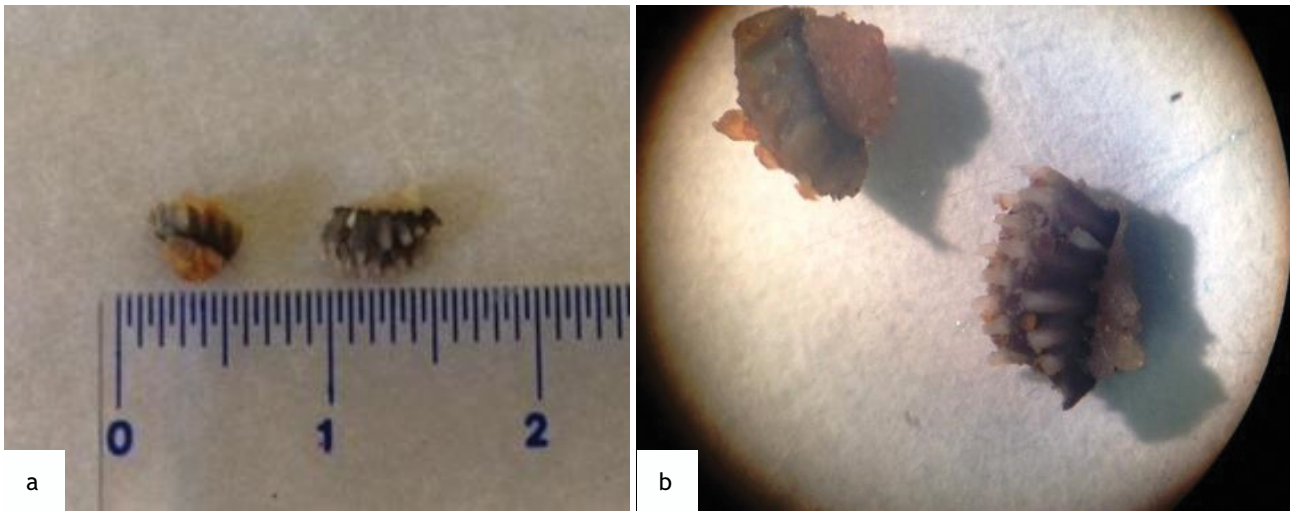


**Figure 2.** Size of the taste bud fragments found in the product (meat pasty), as observed by the naked eye (a) and under a stereoscopic microscope (b) under 30x magnification.

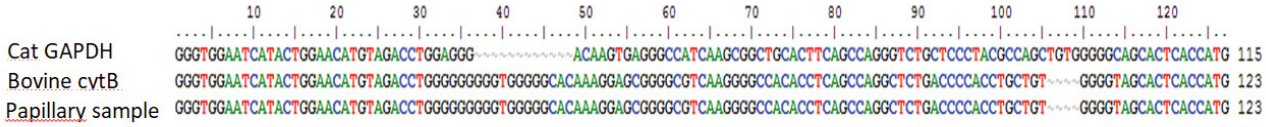


**Figure 3.** Nucleotide alignment of partial cat GAPHD and bovine cytochrome B (cytB) genes and the sequence obtained after PCR for the papillary sample, evidencing its identity with bovine.

### Molecular analysis

As can be seen in Figure 3, the PCR gene sequence of the taste bud samples revealed homology to a genotype sequence of the bovine cytochrome B (cytB) genotype available from GenBank. For the alignment, the Clustal W program of BioEdit version 7.0.9 (1997-2007, Tom Hall, Ibis Biosciences Carlsbad, CA) was used.

## DISCUSSION

The macroscopic analysis identified inferior tissues (taste buds) used as a part of the bovine meat pasty filling, and PCR confirmed the bovine origin of this material, indicating no relation with feline species.

According to the RIISPOA, Decree n° 9.013/2017[4], the use of inferior tissues of animal origin is an unlawful and fraudulent action as well as a bad hygienic and food manufacturing practice[13], requiring notice and appropriate actions from the local Food Health Surveillance.

Complaints of inappropriate and suspicious conditions by consumers is an important practice that helps official agencies identify irregularities and risk factors for the population[14], taking appropriate measures involving educational, punitive, seizure, interdiction, and risk communication actions, at the local, regional, and national levels[15].

PCR has proved to be a useful tool in the detection and characterization of foreign matter in food and water. However, despite the availability of different methods to obtain nucleic acids, the complexity of the different food matrices makes it difficult to extract the genetic material for analysis. Accordingly, different extraction methods have been developed and tested for application in the laboratory routine, either for the identification of genotypes in food components or for the investigation of foreign matter. In this study, the use of trypsin and versene (ATV) in the sample pre-treatment allowed a good extraction performance, aiding in the digestion of the tissue and ensuring reliable results and confirming the presence of bovine DNA and absence of feline DNA.

Achieving success in this case was possible owing to the synergistic performance of the Health Surveillance of Itapira/SP, the IAL, and the Labmas of the VPS of the FMVZ/USP, which by applying their respective specialties allowed the consumer to be informed before the complaint and confirmation of the illegal action of the producer. This report is a practical example demonstrating that technical knowledge applied together with the actions of the official services dedicated to safeguarding public health, enables the State to fulfill its role of guaranteeing human right and dignity from safe and healthy food.

In a review, Salete et al. (2018) showed that the most prevalent types of adulteration in Brazil were intentionally dilution and substitution, in order to obtain economic advantages. An example in meat products is the substitution of animal or vegetable species for similar ones with less economic value. Milk and dairy products and vegetable oils had the highest prevalence and incidence of adulterations in Brazil from 2007 to 2017. However, a large-scale of food fraud and adulteration incidents are reported in the scientific literature, but smaller incidents are typically only reported in the media. In addition, the underreporting of food fraud and adulteration are common, especially when the adverse health effects are chronic with unclear evidence of cause and effect. Food fraud and adulteration deliberately designed to evade detection is also difficult to report in academic journals[16].

## CONCLUSIONS

As established by the current national legislation, the presence of bovine taste buds in ready-to-eat meat pasty is a fraud caused by the improper use of inferior tissues as raw material in the preparation of minced meat.

Microscopic analysis allowed the identification of taste buds, and PCR confirmed the bovine origin of the taste buds found in meat pasty samples obtained for orientation analysis and inspection purposes, based on a consumer complaint.

The ordered and integrated action of different technical expertise allowed prompt and efficient action in the elucidation of foreign material in ready-to-eat food (meat pasty) from the case of a consumer complaint in the municipality of Itapira/SP.

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## Conflict of interest

The authors declare that they have no conflicts of interest.