Food Control 84 (2018) 339–344

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Complementary molecular methods detect undeclared species in sausage products at retail markets in Canada

Amanda M. Naaum [a, b, *], Hanan R. Shehata [a, b, c, **], Shu Chen [d], Jiping Li [d], Nicole Tabujara [d], David Awmack [e], Cyril Lutze-Wallace [e], Robert Hanner [a, b, ***]

[a] Department of Integrative Biology, University of Guelph, Ontario, Canada
[b] Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada
[c] Microbiology Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt
[d] Laboratory Services Division, University of Guelph, Guelph, Ontario, Canada
[e] Canadian Food Inspection Agency Food Safety Science Directorate, Science Branch, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form
27 July 2017
Accepted 29 July 2017
Available online 31 July 2017

Keywords:
DNA barcoding
ddPCR
qPCR
Meat mislabeling
Sausage
Meat species quantification

ABSTRACT

Accurate food labelling is of utmost importance for food safety and consumer choice in the food chain. Complete or partial substitution, whether intentional or unintentional, may introduce food pathogens or allergens to a product or affect personal or religious beliefs. Several studies around the world have reported different degrees of species substitution in meat products but no similar studies have been conducted in the Canadian market for sausage products. In this study, 100 raw meat sausage samples that were labelled as single meat species products (beef, pork, chicken or turkey) were collected from retail establishments across Canada and were surveyed for the presence of a panel of non-labeled species. The predominant meat species were determined using DNA barcoding and contaminant or unclaimed meat species were detected using digital droplet PCR using species specific primers and probes. All samples were also tested for presence of horse meat using real-time PCR. All samples contained the predominant species matching the label species except for five turkey sausage samples which contained chicken as the predominant species. Second, this analysis showed that 6% of beef sausages also contained pork, 20% of chicken sausages contained turkey while 5% contained beef, and 5% of pork sausages also contained beef. Five samples labeled as turkey sausage contained no turkey and one pork sample was found to contain horse meat. The overall mislabeling rate detected in this study was 20% and the results provide a baseline for assessing species mislabeling in processed meat products in Canada.

1. Introduction

Accurate food labelling is important to ensure food safety and quality management in the supply chain, as well as to support consumer choice. Substituted species, whether trace adventitious contaminants or a product of economically motivated adulteration (EMA), may introduce toxins, pathogens, or allergens into products (Spink & Moyer, 2011). Public safety measures and testing procedures are put into place by regulatory agencies based on the declared product contents. Consumers may also choose products labeled free from certain species for personal or religious reasons. The availability of reliable testing methods helps to identify and address issues of undeclared meat species. For example, the increased and improved testing for horse in meat products throughout Europe has brought light to, and helped mitigate, one of the largest food scandals in recent history (O'Mahony, 2013). Food authenticity tests often target DNA as it is more robust to processing associated with many food products, in contrast to protein-dependent methods (Ballin, 2010). Various DNA-based methodologies can be employed to identify the component species of meat products.

Studies of species identification of commercial meat products have been conducted using DNA-based methods in several

* Corresponding author. Department of Integrative Biology, University of Guelph, Ontario, Canada.
** Corresponding author. Department of Integrative Biology, University of Guelph, Ontario, Canada.
*** Corresponding author. Department of Integrative Biology, University of Guelph, Ontario, Canada.
E-mail addresses: anaaum@uoguelph.ca (A.M. Naaum), hshehata@uoguelph.ca (H.R. Shehata), rhanner@uoguelph.ca (R. Hanner).

http://dx.doi.org/10.1016/j.foodcont.2017.07.040

countries including Thailand (Kitpipit, Sittichan, & Thanakiatkrai, 2014), Turkey (Ulca, Balta, Çağın, & Senyuva, 2013), Iran (Doosti, Dehkordi, & Rahimi, 2014; Mehdizadeh et al., 2014), the United States (Kane & Hellberg, 2016; Quinto, Tinoco, & Hellberg, 2016), South Africa (Cawthorn, Steinman, & Hoffman, 2013; D'Amato, Alechine, Cloete, Davison, & Corach, 2013), Spain (Rodríguez et al., 2003), China (Cai et al., 2014), Germany and the Netherlands (Floren, Wiedemann, Brenig, Schutz, & Beck, 2015; von Bargen, Brockmeyer, & Humpf, 2014), the United Arab Emirates (Premanandh, Sabbagh, & Maruthamuthu, 2013) and Ireland (O'Mahony, 2013). Many studies focused heavily on processed meat products like ground meat or sausage where the opportunity for mislabeling is higher. These studies have revealed levels of mislabeling of up to 70%, where products are either wholly or partially substituted with species not listed on the label including pork, horse, chicken and others. Although there are examples where little or no mislabeling was found, products with undeclared ingredients were found in almost all published studies.

DNA barcoding is one method that has particular promise for species identification in food (Galimberti et al., 2013). For animal species, this method involves sequencing of a ~650bp fragment of the COI gene (Hebert, Ratnasingham, & de Waard 2003). While used successfully to identify mislabeling of meat products (D'Amato et al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016), it cannot be reliably used on its own to identify species in mixtures and can only detect whole substitution. However, the extensive availability of DNA barcode sequences for commercial and game meat species allows for development of other types of testing that require DNA sequence information for development of primers and probes. This includes droplet digital PCR (ddPCR), which can identify the presence of undeclared species from a mixture and also quantify the amount of that species, allowing separation of cases of adventitious presence from adulteration. Shehata et al., 2017 have developed a method for quantitative ddPCR detection of turkey, chicken, pork and beef which can be used to test the contents of market samples.

Despite the fact that ingredient species must be listed in Canadian food, a national market survey looking at incidence of mislabeling in sausage meat products has not been conducted. In this study, we provide a baseline for occurrence of species mislabeling in sausage products purchased on the Canadian market using a combination of DNA-based methods, including DNA barcoding, digital PCR and real-time PCR. This tiered approach allows the identification of whole substitution using DNA barcoding, as well as identification of undeclared species from a panel of ddPCR assays targeting pork, beef, chicken or turkey (Shehata et al., 2017) and one horse-specific real-time PCR assay. The ddPCR approach also provides a measure of the level of adulteration to differentiate cases that are likely purposeful, from adventitious cases.

2. Materials and methods

2.1. Sample collection

Samplers under contract to the Canadian Food Inspection Agency (CFIA) were utilised to collect a total of 100 sausage samples in three major Canadian cities (Montreal n = 40, Toronto n = 25 and Calgary n = 35) at various retail locations (Table 1). All sampling took place between January 17 and February 25, 2016. Products were purchased from national grocery store chains, regional and local grocery stores, and speciality stores. Sausages were labeled as containing only pork, beef, chicken or turkey (pork n = 38, beef n = 27, chicken n = 20, and turkey n = 15) and did not include more than a single source animal species as identified on the ingredients list. Wieners ("hot dog"), Vienna sausages and sausages containing cheese were excluded from the survey. Sausages were raw, fresh or previously frozen, ready-to-cook, in casings that were prepackaged, or partitioned in-store. No bulk sausage meat samples (without casings) were selected for this study. Digital images of labels and packaging were taken at the time of submission to the laboratory along with details of purchase, and submitted electronically to the CFIA office. Samples were shipped directly to the laboratory in coolers with cold packs. Ninety samples originated from Canada while five samples were imported from the USA and five samples were from unknown origin.

2.2. Sample preparation and controls

The sausage samples were removed from casing, cut into ~1.0–1.5 cm pieces, and then ~25–30 g of representative sample pieces combined from every sausage in the package were homogenized in a Cuisinart® grinder for 3–5 min. The grinder was cleaned and treated with 20% bleach to remove residual DNA between samples. The homogenized samples were then used for DNA extraction. An artificial DNA fragment cloned into a plasmid was used as an internal control to monitor ddPCR procedures (Shehata et al., 2017). For ddPCR, pure bovine, pork, chicken and turkey muscle meat tissues were used as reference materials. For each of the four meat species, tissue of equal weight from five representative single meat species sausage samples, as verified by DNA barcoding and species-specific ddPCR assays, were pooled to establish calibration curves between ddPCR output (copies) and DNA amount (ng) in sausages. The standard curves were established at three-fold dilutions between concentrations ranging from 0.0039 to 0.32 ng for beef, pork and chicken (five points in total), and from 0.0013 to 0.32 ng for turkey (six points in total). Fortified samples, prepared by adding 0.1% (DNA by mass) of the target animal species to a non-target animal species, were used as positive controls. A fish tissue sample, reagent blank and sterile water were used as negative controls.

2.3. DNA extraction

A representative portion (~500 mg) of each homogenized sausage sample was used for DNA extraction in the lysis stage, to increase the amount of representation from the full sausage package. The volume of lysis buffer was increased to accommodate this. After lysis, the usual process was followed for extracting genomic DNA using DNeasy Blood and Tissue® Kit (Qiagen, Mississauga, ON) according to the manufacturer's protocol for "Purification of Total DNA from Plant/Animal Tissue". DNA concentrations and quality (A_{260nm} and A_{280nm}) were determined using both NanoDrop ND-2000 UV–Vis Spectrophotometer (Thermo Fisher Scientific, Ottawa, ON) and Qubit® Fluorometer with the Qubit® dsDNA BR Assay Kit (Thermo-fisher Scientific). The DNA samples were diluted to 10–20 ng/μL in AE buffer, and then were either tested directly or were frozen at −20 °C for testing at a later date.

2.4. Identification of the predominant meat species using DNA barcoding

Primers (Table 2) targeting the mitochondrial cytochrome oxidase subunit I (COI) gene were designed using the PrimerQuest Tool www.idtdna.com/Primerquest/Home/Index) based on NCBI sequences for the following species: *Bos taurus* (AF493542), *Sus scrofa* (KP301137), *Gallus gallus* (KM096846), and *Meleagris gallopavo* (JF275060). The accession numbers represent the reference sequences used, however multiple sequences from each species were considered during primer design. Each PCR reaction mix (25 μL) contained 1x HotStarTaq Master Mix (Qiagen), 0.5 μM of each of the