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ORIGINAL ARTICLE

Molecular identification of isolated fungi, microbial and heavy metal contamination of canned meat products sold in Riyadh, Saudi Arabia

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Abstract Several studies have shown that canned meat products may be contaminated with fungal elements, bacteria and even heavy metals which may occur during the transportation, storage and handling processes. We conducted this study to determine the fungal, microbial and heavy metal contents of canned meats in Saudi Arabia. Of the 13 canned meat samples studied, *Aspergillus* and *Penicillium* were found in more than 70% of the total samples. Sequences of *Penicillium* species isolated from meat samples generated a phylogenetic tree which shows that the studied isolates were clustered in four groups. No bacterial contamination was noted in all of the samples. Nine of the 13 samples had iron concentrations above the permissible limit. All samples had zinc and copper levels below the maximum permissible limit. Four samples had cadmium levels above the maximum permissible level. All samples had levels of lead above the maximum permissible levels. These results indicate that fungal elements and higher levels of heavy metals such as lead and cadmium can be found in canned meat products. This may pose as a real danger to consumers, since canned meat products are readily accessible and convenient in Saudi Arabia.

1. Introduction

The market for canned foods in Saudi Arabia has increased over the years, with the canned fish/seafood category leading the canned food market in Saudi Arabia (Market Indicator Report, 2011). Meats, when exposed to biological and chemical contamination, can serve as an excellent culture media for growth of microorganisms, since they are a good source of amino acids and nitrogens (BD Diagnostics Manual, 2009). When these microorganisms multiply in food, they produce toxins that are hazardous and even lethal to humans (Billy and Wachsmuth, 1997). Canning of meat may exacerbate contamination by microorganisms when processing practices are poor, especially in states of low acidity and incubation at temperatures above 37 °C. Contamination may also occur during transportation, storage and handling processes. Several bacterial species known to contaminate canned meats include *Escherichia coli*, *Clostridium*, *Staphylococcus aureus*, *Listeria* and *Bacillus* species (Blake et al., 1977; Cragg and

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Andrews, 1973; Mitrica and Granum, 1979). For one, *Clostridium botulinum* can grow at pH above 4.6 and is able to form very heat resistant spores and a lethal toxin (Brooks, 2012).

Several studies have shown that despite strict regulations on processing and canning of food products, a large number of cases of poisoning are reported primarily due to the consumption of canned foods (Czerwiński et al., 2012). In March 1995, a large outbreak of food poisoning among 188 inmates in an institution occurred in Singapore. The incident strongly implicated imported canned pork, as the most probable cause of food poisoning (Ng et al., 1997). It has been shown that mutagens can be produced during heat-processing of canned foods (Krone and Iwaoka, 1984). Furthermore, canned cured meats are more prone to spoilage and potentially hazardous if they are temperature abused after a period of refrigerated storage (Tompkin et al., 1978).

Besides microbial contamination of canned meats, there is also a widespread concern on heavy metal contamination of these canned meat products. Several studies have shown that canned products may have high levels of lead (Pb), the source of which originates from the solder used in the canning process (Mol, 2011). Other heavy metals particularly mercury (Storelli et al., 2010) and cadmium (Cd) have been broadly studied (Shiber, 2011; Storelli et al., 2010; Gutiérrez et al., 2007). Levels of heavy metals in canned fish have been widely reported (Ashraf et al., 2006; Tahvonen and Kumpulainen, 1996). It has also been shown that storage of canned meat gradually increased the concentration of iron (Fe), copper (Cu), lead (Pb) and zinc (Zn) with time in some canned meats (Arvanitoyannis, 1990).

This study was conducted to quantitatively and qualitatively evaluate the microbial and heavy metal contents of canned meats in Riyadh, Saudi Arabia.

2. Materials and methods

Thirteen (13) samples of canned foods comprising three of each of different brands of canned meat within the expiry date as indicated on the cans were randomly (without specific order) collected from supermarkets and shopping malls within Riyadh City, Saudi Arabia between October and November 2012. Samples were taken to the laboratory for analysis. The information on the container/labels was recorded to include NAFDAC (National Agency for Food and Drug Administration and Control) number, manufacture and expiry dates, batch number, manufacturer's address, preservative(s) and compositions. Cans were examined for evidence of bloating, leakage and physical damage.

Details of these samples are listed in the following table.

Sample number	Type of processed meat samples	Producing company and country
1	Corned beef	Queen's Way, Brazil
2	Corned beef	Americana, Packed in Brazil under authority from Food Industries, Dubai, UAE
3	Chicken luncheon meat	Robert (Hot spiced), Damkjaer, Denmark
4	Chicken luncheon meat	Union, Siniora Food Industries, Jordan
5	Pure Beef Luncheon meat	Freshly. Orient Provision & Trading Co., LTD, Brazil
6	Corned beef	Target, Brazil
7	Chicken luncheon meat	California Garden, Packed in Holland under authority from Food Industries
8	Mortadele de Poulet	Danborg, Robert, Damkjaer, Denmark
9	Corned beef	Bordon, Brazil
10	Corned beef	California Garden, Pampeano Alimentos Hulha Negra/RS, Brazil
11	Vienna canned sausages – chicken brand	Picnic, USA, San Diego, California, Food Industries
12	Beef Luncheon	Siniora Food Industries, Jordan
13	Corned beef	Target, Brazil

Prior to analysis, the surface of the container was cleaned with 70% ethanol and tincture of iodine. Containers were opened near the flame of the Bunsen burner to avoid contamination. The pH of the samples was recorded using a pH meter.

3. Bacteriological analysis

Ten gram portions of the foods was blended in a sterile warring blender and inoculated into triplicate tubes of 90 ml Brain Heart Infusion (BHI) broth (Oxoid) and Cooked Meat medium (Oxoid) and into MacConkey and EMB agar plates that were incubated aerobically at 37 °C and 45 °C for Coliforms. At the end of incubation time, colonies were counted using colony counter (Stuart Scientific, UK). Results were expressed as cfu/g. Characteristic colonies on plates were Gram stained, purified by repeated subculturing and stored on agar slants or agar stab if anaerobic, until further characterization. Identification of isolates was done by Gram staining, indole test, urease test, catalase test, methyl red test, citrate utilization test, Vogues-Proskauer test, gelatin liquefaction, starch hydrolysis, sugar fermentation tests, motility and cultural characteristics on culture media.

Confirmatory identification was based on the following methods; Plain agar for total bacterial count (TBC), Violet Red Bile agar for total and fecal coliform count (FCC), Baird Parker agar for *Staphylococcus* count, *Bacillus cereus* agar for *Bacillus cereus* count, Brilliant green agar for Salmonella count and Rose Bengal agar for Total mold count.

4. Mycological analysis

The dilution plate method described by Pitt and Hocking in 2009 was employed for this purpose (Easa, 2010). Proper dilution rates ranged from 1/2 to 1/10 (weight/volume). Cultures in 5 replicates per sample were prepared and incubated at 28 °C for 7–10 days after which the growing colonies were counted, identified and isolated in pure cultures.