Abstract

In this study, 500 ready to eat raw meat samples (minced meat, lahmacun ingredients, kebap, stew and meatball samples) analyzed for different animal originated DNA residues (pork, chicken, cattle, sheep, horse, donkey, cat, dog, mouse, cockroach and house fly) by PCR procedures. Besides, all the samples were analyzed for important foodborne pathogens (coliforms, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes and Salmonella spp.). According to the results, total of 52 samples were determined as adulterated and different originated animal DNA samples were found (chicken, horse and sheep DNA residues). Adulterated samples were also determined more risky for the consumers in microbiological aspect.

Keywords: PCR, Species identification, Ready to eat meat products, Foodborne pathogens

INTRODUCTION

The composition of food is a major concern of consumers today. In the case of adulterated meat product consumption, several factors including economic, food safety (allergy) and moral reasons (religious belief), trigger such apprehensions. Among these concerns, consumers are most sensitive because of religious factors and do not tolerate even trace amounts of adulteration of meat products with forbidden meats like pork [1]. Hygiene and right labeling notified on the label of any food stuff are very important criteria especially for public health.

This study aimed to examine various meat and meat products (kebaps, lahmacun ingredients, minced meat, stews, various meat balls etc.) which are presented in various sales points (restaurants, butcher shops, groceries etc.) in Istanbul region, to determine their ingredients.
through DNA typing method and to specify the different animal tissues/residuals in these products. Besides, all of the samples are checked for the 6 primary foodborne pathogens which can pose serious microbiological threats for consumers' health. The differences between adulterated and not adulterated products are determined by statistical methods.

**MATERIALS and METHODS**

**Specimen Handling**

Random sampling method has been used in this study. From 500 different sales points in the Istanbul region 500 meats and meat product samples have been collected.

**Microbiological Analyses**

The number of TAB was defined in Plate Count Agar (Oxoid, CM0325), coliforms in VRB (Oxoid, CM1082), *E. coli* in Tryptone Bile X-Glucuronide Medium Agar (Oxoid, CM0945), *S. aureus* in Baird-Parker Agar (Oxoid, CM0275) and DNAse Agar (Oxoid CM0321), *Salmonella* spp. in Xylose Lysine Desoxycholate Agar (Oxoid, CM0469) and Hectoen Enteric Agar (Oxoid, CM0419), and *L. monocytogenes* in Chromogenic Listeria Agar (ISO) Base (Oxoid, PO 5183) and Chromogenic Listeria Selective Supplement (ISO) (Oxoid, SR0226) and Oxford (Oxoid, CM856) and Palcam Agar (Oxoid, CM877) respectively to ISO 16649-2 2001, 4833 2003, 6888-1/A1 2004, 11290-1/A1 2005 and 6579/A1 2006 [2-7].

**PCR**

DNA of all isolates were extracted according to the protocol of the manufacturer (Macherey-Nagel, Nucleospin® Tissue). All the extracts were stored at -20°C until they are used as target DNA for the PCR procedure.

**Statistical Analysis**

In order to study the risk differences among adulterated and non-adulterated samples upon the studied microbiological parameters and to determine the statistical significance of these, Pearson correlation analysis has been used [6].

**RESULTS**

18 (3.6%) of the samples showed chicken DNA, 33 (6.6%) of them showed sheep DNA and 1 (0.2%) of them showed horse DNA. None of them showed pork, donkey, cat, dog, mice, cockroach and fly DNA. The detailed refraction of the results can be seen in Table 1. The positive results have been determined through Real-time PCR procedures.

The microbiological results are given in Table 2. According to coliform bacteria indications 41 (%8.2) of the samples, according to *E. coli* parameter indications 23 (4.6%) of the samples, according to *S. aureus* parameter indicators 29 (5.8%) of the samples, according to *L. monocytogenes* indications 8 (1.6%) of the samples, according to *Salmonella* spp., 3 (0.6%) of the samples have been determined as unfit for human consumption. 70.3% of coliforms, 58.7% of *E. coli*, 72.4% of *S. aureus* and 100% of *Salmonella* spp. and *L. monocytogenes* detections are found in the adulterated samples.

**DISCUSSION**

In many countries, food fraud and adulteration in food products, especially in meat and meat products are done either deliberately in order to increase the profit margin or involuntarily as a result of not following the food safety standards, especially in facilities which process more than one animal species.

The main ingredient of kebab in our country is mutton and many kebab shops prepare their kebabs from a mixture of bovine meat and mutton; however, mixing meat products of different animal species either deliberately or accidentally poses a microbiological threat for the consumers, causes the consumers to consume meat products beyond their information. As a result, the consumer is deceived and retrospective follow-up, which is a very important part of food safety procedures, becomes too difficult. It is possible that especially the products containing different types of meat are deliberately adulterated or the facilities producing these in deliberately mingle different meat products.

<table>
<thead>
<tr>
<th>Region</th>
<th>Sample (raw)</th>
<th>Sales Point</th>
<th>Extraneous DNA</th>
<th>DNA Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Istanbul Europe - Istanbul Asia</td>
<td>Lahmacun ingredients</td>
<td>Kebab shop</td>
<td>Chicken</td>
<td>11</td>
</tr>
<tr>
<td>Istanbul Europe - Istanbul Asia</td>
<td>Minced meat</td>
<td>Butcher shop</td>
<td>Chicken</td>
<td>5</td>
</tr>
<tr>
<td>Istanbul Europe</td>
<td>Kebab</td>
<td>Kebab shop</td>
<td>Chicken</td>
<td>2</td>
</tr>
<tr>
<td>Istanbul Europe - Istanbul Asia</td>
<td>Kebab</td>
<td>Kebab shop</td>
<td>Sheep</td>
<td>30</td>
</tr>
<tr>
<td>Istanbul Europe</td>
<td>Minced meat</td>
<td>Butcher shop</td>
<td>Sheep</td>
<td>3</td>
</tr>
<tr>
<td>Istanbul Asia</td>
<td>Minced meat</td>
<td>Butcher shop</td>
<td>Horse</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td></td>
<td>52</td>
</tr>
</tbody>
</table>
Medical literature states that some strains such as *S. aureus* are not very competitive and if their initial counts are lower, they cannot develop properly and their development is easily depressed in mixed cultures. Besides, lactic acid bacteria in the microflora of fermented foods and the antimicrobials they produce like the lactic acid, hydrogen peroxide and bacteriosin suppress pathogens such as *E. coli*, *S. aureus*, *L. monocytogenes* and *B. Cereus*. It is thought that the staff hygiene practices are deficient in the facilities from which the *S. aureus* positive samples have been collected and this is the primary reason of these results.

The adulteration practices pose another risk which is often overlooked but actually important, that is food intolerance. The exogenous substances which are mixed in the adulterated products and the ingredients which might be different from the label information may cause the consumers to develop food intolerance reactions. This is considered one of the main risks of adulteration. Food intolerance may have various reasons. The prevalence of food intolerance reactions against foods and food additives is much higher than food allergies which include an immunological mechanism. Whatever the reason of the adulteration maybe, it results in deficient hygiene conditions and this is a serious threat for the facility, staff and product and consumer health. Besides, microorganisms which reproduce in meat and meat products because of hygiene deficiency can quickly develop single or multi resistance to antibiotics through complex genetic interactions. Our study shows that adulterated products pose a statistically meaningful higher risk for consumer health than unadulterated products.

### REFERENCES