Quality indices of Fresh and Frozen Oriental sausage

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Abstract
A total of 100 of fresh and frozen oriental beef sausage samples (50 of each) were collected from local markets and analyzed for their quality indices through bacteriological and chemical examinations. The bacteriological profile of fresh sausage samples revealed that the mean Log values of Psychrotrophic, Enterobacteriaceae and Coliforms count were 7.164 ±0.131, 5.393 ±0.140 and 5.2308 ±0.1504 log10 CFU/g., respectively. While the mean log values for frozen sausages were 3.404 ±0.589, 3.193 ±0.199 and 3.2973 ±0.2385 log10 CFU/g respectively. Salmonella were detected in 10 (20%) of fresh oriental beef sausage, meanwhile it could not be detected in all examined frozen sausage. The chemical examination showed that the mean values of pH, Total Volatile Nitrogen (TVN mg/100g) and Thiobarbituric acid value (TBA mg malondhyde /kg) were 5.480±0.066, 8.263±0.348 and 0.2346 ±0.0078; 6.503±0.067, 4.399 ±0.082 and 0.4064 ±0.0348 for fresh and frozen and sausage samples respectively.

Keywords: Oriental Sausage, Psychrotrophic, Enterobacteriaceae, Coliforms, Salmonella, PH, TVN, TBA.

Introduction
Meat products are one of the most delicious and popular foods, as they are a vital source of animal protein, essential amino acids, fat, minerals, vitamins and other nutrients (Zafar et al., 2016). Meat products are thought to provide a suitable medium for the growth of a variety of microorganisms because of large proportion of nitrogenous compounds, their high moisture, abundant supply of minerals, a number of fermentable carbohydrates and favorable pH for the majority of microorganisms causing their spoilage, economic losses, foodborne related infections in human and health hazard (Aymerich et al., 2008). Oriental beef sausage is very popular meat product items due to its quick preparation and it also solve the shortage issue of high-priced fresh.
meat that may be out of reach to limited income families (Saad et al., 2018b). Microorganisms contaminate sausage through spices, raw meat and other components, also from equipments, environment and handlers throughout processing influence the microbiological profile of the sausage, (Sachindra et al., 2005). These microorganisms differ with the manufacture method, quality of sausage additives, and contamination degree throughout the processing chain, packaging and storage (Borch and Arinder, 2002). Regarding frozen sausage, freezing is a method for increasing the shelf life of meat products and protects it from being rapid spoilage. It has been practiced for many years to maintain its quality throughout storage, distribution and marketing (Abdel-Aziz 2000). Bacterial load and sanitary measures throughout meat manufacture and unsuitable storage conditions for frozen meat products can be assessed by the total Psychtrophic, Enterobacteriaceae and total Coliforms counts (Hamed et al., 2015).

Psychtrophic bacteria are the main cause of spoilage of meat products which are kept under refrigeration temperature due to their ability to grow at low temperature. Their count can provide useful information about the keeping quality of some meat products (Mousa et al., 2014). The Enterobacteriaceae group has an epidemiological significance as some numbers of its members are pathogenic and might cause serious infections and food poisoning. Furthermore, during the absence of coliforms the total number of Enterobacteriaceae is considered as a sign of possible enteric contamination, (Mercuri et al., 1978). The incidences of Coliform bacteria in examined food samples indicate contamination by animal or human fecal throughout meat products and meat processing chain. They are not themselves pathogenic but are inhabitant of the digestive systems of animals, and thus plentiful in feces (Hamza and Elshrek 2019). Salmonellosis is a worldwide health issue, and considered the second foremost bacterial food-borne gastroenteritis. Despite the fact that Salmonella has over 2,500 different serotypes, only a few of these serotypes have been associated to food-borne diseases on a regular basis (Mahmoud 2012).The Total Volatile Nitrogen (TVN) content is extensively used as a benchmark for microbial decomposition of protein and also throughout the storage period, tissue proteolytic enzymes continue in breaking down the proteins (Gibriel et al., 2007). Lipid oxidation occurs when pro-oxidant agents exceed the antioxidant compounds and factors naturally or deliberately added to meat products. A number of oxygenated products are generated during lipid oxidation as hydroperoxides and peroxyl which may cause major impacts in the
characteristics of meat products throughout storage period. The TBA assays indicate the level of the secondary products resulted from lipid oxidation (Lorenzo et al., 2018). In recent years, consumer awareness has risen for bacteriological quality of sausages. Bacteriological and chemical criteria are a good approach to learn more about the quality and safety of oriental sausage. Therefore, this study was conducted to evaluate the bacteriological and chemical quality of fresh and frozen oriental beef sausages samples collected from local markets, via determination of Psychrotrophic, Enterobacteriaceae, Coliforms count and assessment of pH, TVN and TBA.

Material and methods
1 Collection of samples
A total of one hundred samples of fresh and frozen oriental beef sausages (fifty of each) collected from local markets. The fresh samples were transferred without delayed to the Food Hygiene laboratory, Faculty of Veterinary Medicine, Suez Canal University where prepared for the microbiological evaluation. Frozen samples were thawed in the refrigerator overnight at 4°C.

2 Preparation of samples APHA (2002)
Twenty-five grams from each sample were transferred under aseptic condition to a sterile polyethylene bag containing 225mL of sterile buffered peptone water 0.1%. The bag content was then homogenized using stomacher (Lab. Blender 400, Seward Lab, London) to have a dilution of 10⁻¹. From the original dilution, 1 ml was aseptically transferred to a test tube containing 9 ml sterile buffered peptone water (w/v) 0.1% to prepare a dilution of 10⁻², then from which further tenth fold serial dilution up to 10⁻⁷ were prepared to cover the expected range of sample contamination which could be easy counted.

3 Bacterial analysis:
3.1 Determination of Psychrotrophic count as described by (APHA, 2002).
3.2 Determination of Enterobacteriaceae count as described by (ISO 21528-2).
3.3 Detection of Salmonella as described by (ISO 6579:2002).
3.4 Determination of Coliform count as described by (ISO/FDIS 4832:2005)

4 Chemical analysis:
4.1 Determination of PH as described by (AOAC, 2005).
4.2 Determination of TVN as described by (AOAC, 2005).
4.3 Determination of TBA as described by (AOAC, 2005).

Results and Discussions
Food borne illness caused by consumption of contaminated food with pathogenic bacteria and with their toxins has been of critical concern to public health. More than 250 diverse food borne diseases have been mentioned, and the
bacteria are the causative agents of two thirds of foodborne disease outbreaks (Olsen et al., 2000).

1. Microbiological analysis:

1.1 Psychrotrophic count of sausage:

Due to the increased usage of frozen food nowadays to save time, it is very vital to study the effect and role of Psychrotrophic bacteria in frozen foods spoilage (Kraft, 1992). The data recorded in Table (1) revealed that the mean value ±SE of Psychrotrophic count of the examined fresh sausage was 7.164 ±0.131 (log_{10} CFU/g). While in frozen sausage in Table (2) it was 5.633 ±0.118 (log_{10} CFU/g). The recorded result for fresh sausage was nearly compatible with (Araújo et al., 2018). The recorded result for fresh sausage was relatively higher than that obtained by Bostan and Mahan (2011) and Ali et al. (2021). For frozen sausage, the obtained results matched that to the result obtained by Sharoba (2009) and Badr and Mahmoud (2011), while these results were lower than those results found by Bostan and Mahan (2011). On the other hand, these results were relatively higher than that obtained by Gaafar et al. (2014), Mousa et al., (2014) and Shaltout (2017). The differences in the results were ascribed to the quality of the raw materials used and the application of good manufacturing practice.

1.2 Enterobacteriaceae count sausage

The occurrence of Enterobacteriaceae act as an indicator of food sanitation and it has received an attention of most scientists. The presence of Enterobacteriaceae indicates the potential of toxigenic bacteria and microbiological in meat that leads to public health hazard (Mira, 1989). The data recorded in Table (1) revealed that the mean value ±SE of Enterobacteriaceae count of the examined fresh sausage was 5.393 ±0.140 (log_{10} CFU/g), while in frozen sausage in Table (2) was 3.193 ±0.199 (log_{10} CFU/g). The recorded result for fresh sausage was nearly similar to Al-Mutairi (2011) and Badr and Mahmoud (2011), meanwhile these results were relatively lower than those results found by Oluwafemi and Simisaye (2006), meanwhile the results were relatively higher than those results found by Shaltout et al. (2016b), Salem et al. (2018) and Youness (2018). For frozen sausage, the obtained results almost matched that result obtained by Khalafalla and El-Sherif (1993) and Gaafar et al., (2014). On the other hand, these results were relatively lower than those results found by Badr and Mahmoud (2011). The results were relatively higher than those results found by Mousa et al. (2014) and Shaltout (2017).

1.3 Detection of Salmonella in sausage:

The absence of Salmonella in the meat product samples indicate the
quality of raw meat and other hygienic processing including the quality of the water used in processing (Datta et al., 2012). The data recorded in Table (3) revealed that the incidence of salmonella in the examined fresh sausage was 20%, while in frozen sausage all samples were negative for salmonella. The obtained results for fresh sausage was nearly similar to this reported by Moustafa et al. (2014), Abd El Tawab et al. (2015) and Kayed (2020), while it was relatively lower than results obtained by Hamed et al. (2007), Elhag et al. (2014) and Humaed (2014). On the other hand this result was higher than results obtained by Manihuruk et al. (2017), Younes et al., (2019), and Gamal et al., (2020). For frozen sausages none of the examined samples contained Salmonella, this is in accordance with the results reported by Surkiewicz et al. (1973), Phillips et al. (2006) and Hassanin et al., (2018) Salmonella proved to be highly sensitive to freezing, regardless of the freezing method. On the other hand, it was relatively lower than results obtained by Mousa et al. (2014), Shaltout et al. (2016c) and Saad et al. (2018b). By comparing this result with Egyptian Standard (ES 2005), it revealed that 100% of frozen sausage samples were compatible to Egyptian Standard.

1.4 Coliform count of sausage

The occurrence of coliforms in food indicates poor hygienic standards (Shaltout 2017). The data recorded in Table (1) revealed that the mean value ±SE of Coliform count of the examined fresh sausage samples was 5.2308 ±0.1504 (log_{10} CFU/g), while in frozen sausage samples in Table (2) was 3.2973 ±0.2385 (log_{10} CFU/g). The obtained results for fresh sausage were nearly similar to Oranusi and Braide (2012), Elhadi et al. (2017) and Saad et al. (2018b). On the other hand, these results were relatively lower than result found by Abomengeal (2010), while these results were relatively higher than result obtained by Shaltout et al. (2016a), Hamza and Elshrek (2019) and Younes et al. (2019) For frozen sausage, the obtained results almost matched that result obtained by Shaltout et al. (2016c) and Shaltout (2017), while these results were relatively higher than result found by Sharoba (2009) and Shaltout (2017). On the other hand, these results were relatively lower than result obtained by Hamed et al. (2007), Hassanin et al. (2018) and Saad et al. (2018b). By comparing this result with ES (2005), it revealed that 80% of frozen sausage samples were not compatible to Egyptian organization for standardization and quality control.

2- Chemical analysis:

The data recorded in Table (4) revealed that mean value ±SE of pH of the fresh sausage was 5.480 ±0.066, while in frozen sausage was 6.503 ±0.067. The recorded result
of pH for fresh sausage was nearly similar to Elhag et al. (2014), Manihuruk et al. (2017) and Ali et al. (2021). On the other hand, these results were relatively lower than result found by Humaeda (2014), Slima et al., (2017) and Araújo et al., (2018). For frozen sausage, the obtained results almost matched that result obtained by Hamed et al. (2007), Sharoba (2009) and Badr and Mahmoud (2011). The data recorded in Table (4) revealed that mean value ±SE of TVN of the examined fresh sausage was 8.263 ±0.348, while in frozen sausage was 4.399 ±0.082 mg/100g. The recorded result of TVN for fresh sausage was nearly lower than results reported by Hamed et al. (2007), Ali et al. (2010), Ibrahim (2012) and Ali et al., (2021). For frozen sausage, the obtained results were nearly lower obtained by Hamed et al. (2007) and Sharoba (2009). By comparing this result with ES (2005), it revealed that 100% of frozen sausage samples were compatible to Egyptian organization for standardization and quality control. The data recorded in Table (4) revealed that the mean value ±SE of TBA of the fresh sausage was 0.2346 ±0.0078, while in frozen sausage was 4.399 ±0.082 Mg malondhyde /Kg. The recorded result of TBA for fresh sausage was nearly similar to Ali et al., (2010) and Ali et al., (2021). On the other hand, these results were relatively lower than result found by Hamed et al. (2007), Ibrahim (2012) and Abdel-rasoul (2021). By comparing this result with ES (2005), it revealed that 100% of frozen sausage samples were compatible to Egyptian standard.

**Conclusion:**
Fresh and frozen sausages are considered a hazard source for public health due to presence of Enterobacteriaceae, Salmonella and Coliform this may be ascribed to lack of hygienic conditions, absence of quality control and multi contamination sources throughout sausage processing, packaging, storage and distribution. The undesirable level of contamination which might have been acquired from the environment and surrounding and considered was considered as major cause of spoilage of meat products. Additionally, retailed Egyptian sausage might pose a possible health hazard, making it essential to apply sanitary measures during its processing, handling, storage, packaging, distribution and selling. Therefore, improving the microbiological quality and increasing the shelf life of sausage is necessary by implementation of the principles of Good Manufacturing Practices throughout the chain of sausage manufacturing process.
### Table (1) Statistical analytical results of (log10 CFU/g) of (Psychrotrophic, Enterobacteriaceae and Coliforms) of Fresh sausage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Psychrotrophic</th>
<th>Enterobacteriaceae</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>6.068</td>
<td>3.869</td>
<td>3.6435</td>
</tr>
<tr>
<td>Maximum</td>
<td>8.391</td>
<td>6.146</td>
<td>5.9542</td>
</tr>
<tr>
<td>Mean</td>
<td>7.164</td>
<td>5.393</td>
<td>5.2308</td>
</tr>
<tr>
<td>SE</td>
<td>± 0.131</td>
<td>± 0.140</td>
<td>± 0.1504</td>
</tr>
</tbody>
</table>

### Table (2) Statistical analytical results of (log10 CFU/g) of (Psychrotrophic, Enterobacteriaceae and Coliforms) of frozen sausage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Psychrotrophic</th>
<th>Enterobacteriaceae</th>
<th>Coliforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>4.544</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Max.</td>
<td>6.681</td>
<td>4.041</td>
<td>4.6990</td>
</tr>
<tr>
<td>Mean</td>
<td>5.633</td>
<td>3.193</td>
<td>3.2973</td>
</tr>
<tr>
<td>SE</td>
<td>± 0.118</td>
<td>± 0.199</td>
<td>± 0.2385</td>
</tr>
</tbody>
</table>

### Table (3) Prevalence of salmonella in Fresh sausage.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>%</th>
<th>Negative</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
</tr>
</tbody>
</table>

### Table (4) Statistical analytical results of (pH, TVN and TBA) of Fresh and frozen sausage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fresh Sausage samples</th>
<th>Frozen Sausage samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>TVN mg/100g</td>
</tr>
<tr>
<td>Min.</td>
<td>5.03</td>
<td>6.3</td>
</tr>
<tr>
<td>Max.</td>
<td>5.9</td>
<td>10.500</td>
</tr>
<tr>
<td>Mean</td>
<td>5.480</td>
<td>8.263</td>
</tr>
<tr>
<td>SE</td>
<td>±0.066</td>
<td>±0.348</td>
</tr>
</tbody>
</table>
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