Examination of microbiological parameters relevant to the implementation of GHP and HACCP system in Greek meat industry in the production of cooked sausages and cooked cured meat products

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Abstract

Good hygienic practices (GHPs) and a hazard analysis critical control point (HACCP) system have been started to implement in a preliminary way to the Greek meat industry. Microbiological examination of 349 samples related to general hygiene control points and the critical control points of the system, as a part of an external audit in three meat plants was conducted. Samples of water, raw materials, additives and spices as well as samples of batters and final products were examined. The hygienic of the production personnel and the processing areas was also tested. In conclusion, the application of GHP and HACCP system could be considered adequate but more efforts is demanded toward the control of the microbiological quality of the incoming materials and processing.

Keywords: GHP; HACCP verification; Audit; Cooked sausages; Cooked, cured meat products; Meat hygiene

1. Introduction

Codex Alimentarius defines food hygiene as ‘all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain’. Pre-requisite hygiene programs (good hygiene practices, GHP) and safety assurance systems such as hazard analysis and critical control point (HACCP) are compulsory in EC member states.

In Greece, food plants have started to apply safety programs the past few years in a preliminary way to comply with the food safety legislation. Among the verification activities in plants encompass sampling for monitoring GHP and CCPs and determination of microbiological variables, review of records, flow diagrams and HACCP plan.

Nevertheless, the implementation of such assurance systems and prerequisite programs like GHPs are applied. Among these plants are the meat and sausage industries. Aim of this work is the description of the essential critical control points and general hygiene control points from three meat industry processing lines, one for the production of cooked sausages (e.g. frankfurter style) and the other for the production of cooked cured meat products (e.g. ham), and the results obtained by evaluating certain microbiological parameters in these points.

2. Materials and methods

2.1. Product preparation

2.1.1. Cooked sausages

Cooked sausages was mainly produced by defatted pork meat and/or mechanical debone meat of turkey (MDM), pork lard, collagen, water, spices, flavour enhancers, and preservatives (like salt, nitrates, and ascorbate), soya flour, dried skim milk and starch were used. All the ingredients were mixed in the cutter machine and stuffed in casings mainly made of collagen.
Frozen defatted meat and/or MDM first was weighed and then was added with collagen into the cutter machine. Seasonings and ingredients were followed with cooled water or flake ice. With the machine operating frozen pork lard was added and lastly the starch. The whole operation was carried out in appropriate chopping speed and time until emulsion was produced. During processing the temperature into the cutter did not exceed 12 °C. The emulsion was stuffed in casings of different size. Then the stuffed product was placed in ovens in which they were dried, smoked and finally cooked. The sausages were heated until the core temperature was raised at 72 °C. After thermal processing sausages were rapidly cooled down at a temperature of 35 °C. The temperature decrease was carried out by water. The finished product was stored at refrigerators (0-4 °C), and packed under vacuum or modified atmosphere in the packaging room prior to its distribution or sell. What it concerns frankfurter style sausages, these were peeled before packaging. Fig. 1 shows the flow diagram for the preparation of cooked sausages.

### 2.1.2. Cooked cured meat products

They were prepared from a wide variety of cuts of meat. For example, for cooked ham was used boneless pork from the hind leg cut into large muscle pieces while for cooked bacon was used boneless pork from the belly. The cure was contained nitrite, salt and other components such as ascorbate, phosphate, antioxidants, spices and sugar.

After butchering to remove fat and rinds unfrozen meat was mechanically pumped with brine. In each in-

![Flow diagram of the preparation of cooked, cured sausages.](image-url)
cision a quantity of brine was injected under pressure (1.5–2.0 atm) directly into the muscle tissue. During pumping the temperature was held at 2 °C while that of the brine at 1–4 °C. After brining the meat was transferred into tumbling machine under vacuum and low temperature (0–2 °C). Then, the meat was “massaged” for appropriate time while during “massage” was included short periods of rest. After tumbling, meat was stuffed in casings or nets and was subjected to heat processing. Heat treatment might have included drying and smoking except cooking. Heat treatment finished when a core temperature of 70–72 °C was reached. Then, rapidly cooling down at a temperature of 35 °C was carried out in a period of 15 min by water. The finished product was stored at 0–4 °C, after which it was either sold in whole parts, or cut down in two parts or slices in the cutting room and packed under vacuum or in modified atmospheres. The production line of cooked cured meat is shown in Fig. 2.

2.2. General hygiene control and critical control points

For both, cooked sausages and cooked cured meat products crucial factors for their hygiene and safety were the temperature in the processing lines which must be held below 12 °C and the sanitation, not only of the personnel but also of the equipment and the plant. It is obvious that a good hygiene practice is a prerequisite tool for an effective HACCP system. Moreover it was necessary for all products to avoid moving back and forth to minimize possible cross-contamination and

Fig. 2. Flow diagram of the preparation of cooked, cured meat products.
their movement from one step of the processing line to another had to be done quickly with no delay.

Incoming materials and especially raw meat may support the growth of microbes because they are used in large quantities. For this reason they were consisted as a general hygiene control point (GHCP1) and were examined at each receipt. Certified suppliers and rigorous criteria for raw material with low microbial counts and absence of pathogens could greatly contribute to the hygienic quality and safety of the final product. Food handling errors, inadequate storage practices, and improper holding temperatures may also occur (Gill & Molin, 1991).

**Cooked sausages.** During chopping procedure of all ingredients into the cutter the temperature had to hold below 12 °C (GHCP2). The core temperature during heat processing consists CCP (CCP1). The cooked sausages were heated until they reached an internal temperature well over 72 °C. To avoid the growth of any thermotolerant bacteria or spores potentially present, it was essential to cool the product rapidly after processing and to prevent storage at high ambient temperatures. Reducing temperature from 72 to 10 °C had to be succeeded in 2 h. The time needed to cool down the cooked sausages consist CCP2. Water used to reduce temperature could be a source of contamination and in this way contaminants could be transferred to product surfaces. For this reason it is essential to examine (at least monthly) the microbiological quality of the water. Major sources of contamination were the slicing machines, conveyor belts and personnel-handling product during packing. To avoid any contamination during this final step the plant had to establish high standard of hygiene conditions. Moreover the personnel hygiene had to be the appropriate. During storage at chilling conditions the temperature had to maintain at 4 °C or less in order to ensure the microbiological safety of the cooked products.

**Cooked, cured meat products.** Thawing of frozen meat was succeeded into water baths. To avoid proliferation after resuscitation of injured microorganisms water had to be continuously renewed. In other cases, water must be changed at the appearance of blood fluids (CCP3). It is well known that blood itself is an excellent medium for the bacteria cultivation.

To avoid contamination of thawed meat fresh brine had always to be added. Most of the times old brines contain salt tolerant bacteria. The whole procedure had to be held at low temperature according to good hygiene practice. Additionally the time intervals between brine injection and tumbling had not to be exceeded 1 h.

Cooking, cooling, slicing and packing are critical control points, as described for cooked sausages. More specific, thermal processing and cooling down are CCP4 and CCP5, respectively.

### 2.3. Sampling procedure

**Raw materials.** Frozen raw meat samples about 100 g each were randomly taken directly from the factory’s freezer warehouse, while they were still in unopened packs as received from the suppliers. The samples were removed aseptically by using sterile knives. All samples of raw materials were placed in pre-sterilized bags, transported to the laboratory on gel-ice in isothermic containers stored at 4 °C and analyzed within 18 h. Granular or powder samples (starch, salt, spices, etc.) were placed in screw-capped sterile containers and transported without refrigeration.

**Batters and final products.** Samples were taken from the cutter (100 g of finished batter) before stuffing and transported under refrigeration (0-4 °C) to the laboratory in sterilized screw-capped containers. Samples were analyzed on their arrival. Final products from the same runs were randomly collected after packaging. Similarly, these samples were analyzed on their arrival after the removal (aseptically) of the casing or the plastic film. Cooked cured meat products were examined after heat processing.

**Water.** Samples (approx. 150 ml) from 10 different outlets were taken from each plant and transferred to the laboratory in pre-sterilized screw-capped bottles containing Na2S2O3 (5%) under refrigeration (0-4 °C) into isothermic containers. Samples were analyzed on their arrival.

**Personnel and equipment sanitation.** Sterile cotton swabs (Vive) were used to sample the personnel at the processing lines and the surfaces of various processing equipment (like teflon tables, cutters, tumblers, slicers, etc.). Except of the personnel swabbed during working hours, all swabs were taken at the end of the day, when the routine cleaning and sanitizing practices had been completed. The swabbing area was approximately 20 cm². If the surface was dry, swabs were pre-moistened with 1/4 Ringer solution. Swabs were transported to the laboratory tubes containing 10 ml of 1/4 Ringer solution (Oxoid), kept at 4 °C and were analyzed on their arrival.

### 2.4. Microbiological analysis

For microbiological analysis, an analytical unit (25 g) was aseptically taken from each sample unit, added to 225 ml of sterile 0.1% (w/v) peptone water (Oxoid) and homogenized in a stomacher 400 (Seward A.J., London, England) for 2 min at medium speed and room temperature. For spices and additives analysis an analytical unit (10 g) was used. Serial decimal dilutions in 1/4 Ringer solution (Oxoid) were prepared and 1 or 0.1 ml samples of appropriate dilutions were poured or spread in duplicate on selective or non-selective agar plates.

**Enumeration of total viable counts (TVCs).** Total viable counts were determined by spreading plate method.
on plate count agar (PCA, Merck). Plates were incubated at 30 °C for 48 h. All colonies were counted.

Enumeration of glucose fermented enterobacteria. Enterobacteria were enumerated by poured plate method in violet red bile dextrose agar (VRBD, Merck), overlayed with 5 ml of the same medium, and incubated at 37 °C for 24 h. Characteristic pink to red-purple colonies of 0.5 mm diameter or more with haloes were counted.

Enumeration of anaerobic sulfite reducing clostridia. For sulfite reducing clostridia tubes containing 20 ml of melted (45 °C) sulfite-polyoxymxin-sulfadiazine agar (SPS, Merck) were inoculated with 10 ml from the first dilution (equal to 1 g of product), cooled rapidly in ice-water and overlayed with 2 ml of sterile paraffin oil to exclude oxygen and were incubated at 37 °C for 24 h. Characteristic black colonies were enumerated.

Enumeration of Staphylococcus aureus. Pathogenic staphylococci were determined by spreading plate method on Baird-Parker agar (BP, Merck). Plates were incubated at 37 °C for 48 h. Staphylococcal colonies showing lecithinase activity on Baird-Parker’s medium were streaked on Nutrient agar (NA, Merck) and rapidly tested for agglutination by the staphyloidal test (bioMerieux sa, Marcy-l’Etoile, France).

Presence–absence test of Salmonella spp. For detection of Salmonella pre-enrichment was carried out by suspending 25 g of sample in 225 ml of Salmosyst Broth Base (Merck) followed by incubation at 37 °C for 6–8 h; selective enrichment was done by transferring 10 ml of pre-enrichment culture into a sterile tube containing one salmosyst selective supplement tablet (Merck). Samples were incubated at 37 °C for 18–22 h prior to being streaked on Rambach agar (Merck) and XLD agar (Merck). After incubation at 37 °C for 48 h the plates were checked for characteristic Salmonella colonies.

Presence–absence of Listeria monocytogenes. For Listeria detection, enrichment was done by suspending 25 g of sample in 225 ml Listeria enrichment broth (Merck) followed by incubation at 30 °C for 48 h, after that the culture was streaked on PALCAM agar (Merck), incubated at 30 °C for 48 h. Typical Listeria spp. colonies were streaked on CASO agar (Merck) and incubated at 37 °C for 24 h. The identity of pure colonies was further confirmed by the API Listeria test (bioMerieux sa, Marcy-l’Etoile, France).

Analysis of water. Serial decimal dilutions in 1/4 Ringer solution were prepared and duplicate 1 ml samples of appropriate dilutions poured on total agar plates. Plates were incubated at 37 °C/48 h and 22 °C/3–5 days. For Enterobacteria detection 100 ml of each sample were filtered by vacuum pump with filter (0.22 μm, Gelman apparatus (model 13156, Gelman Science Ann Arbor, Michigan, US). After filtration, the filter was placed on solidified VRBD-agar (Merck). Plates were incubated at 37 °C for 24 h.

Statistical analysis. One way analysis of variance was conducted for data obtained from enumeration of total viable counts and enterobacteria in the three plants (A–C) and in selected critical control points and general hygiene control points with a statistical software (Systat).

3. Results and discussion

3.1. Raw materials

The CCPs and GHCP that were examined and their critical limits are given in Table 1. For the microbiological evaluation of raw meat materials, 29 samples obtained from the 3 plants. The contribution to the total samples of plants A, B and C was 7, 18 and 4, respectively. The results showed that for the plants A, B and C, 57%, 83% and 100% of the samples were compliant with the reference values (Fig. 3a).

The reason of deviation was the poor hygienic quality of the raw materials (such as pork lard, mince, etc.), inadequate handling and storage practices. Deviation is also derived from co-operation with non-approved suppliers. Selection of certified suppliers and rigorous criteria for raw material with low total counts and absence of pathogens could greatly contribute to the hygienic quality and safety of the product. Fig. 3b shows the percentage of each microbiological parameter and their combination, which cause this deviation in the three plants. It is well known that the hygiene of meat processing depends on the conditions under which animal are reared, slaughtered and processed. Raw meat may support the growth of microbes. The colonization of microorganisms can be further altered by manipulation of the intrinsic, extrinsic and processing factors (Brown, 1982). Food handling errors often contribute to high counts of microorganisms. These errors include factors as improper holding temperatures, inadequate cooking, contaminated instruments used in dressing (knives, saws, cleavers and hooks), various vessels and receptacles and food handler hygiene (NACMCF, 1993).

For the examination of additives and spices, 43 and 51 samples were examined, respectively. All additives (100%) found to be below the reference values (Table 1). On the other hand 25%, 43%, and 100% of the examined spices (Fig. 4a) were found to have microbial populations below the critical limit of plants A, B and C, respectively (Table 1). The high percentage of non-compliant samples was due to the fact that they were not sterilized. Plants A and B showed non-compliance at a percentage of 75% and 57%, respectively. Exception was the plant C, which uses irradiated spices packed under vacuum. The contribution of TVC and enterobacteria to the deviation from the examined parameters as well as
the combination of the above microorganisms is showed in Fig. 4b.

Spices can contain very high levels of microorganisms, in particular spore forming *Bacillus* sp. and more frequently, *Clostridium* sp. Often spices are laid out in fields, sides of roads, or a bank of rives to dry and are subjected to a high degree of contamination. It is not uncommon to have total plate counts on black pepper up to $1.3 \times 10^7$ cfu/g. Even onion powder grown in USA can have counts as high as $3.0 \times 10^6$ cfu/g. Often spices are treated with sterilizing agents to reduce bacterial loads. The two most common treatments are ethylene

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<p>| GHCP-CCP Microbiological variables examined during verification of HACCP systems |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>TVC</th>
<th>Enterobacteria</th>
<th><em>Salmonella</em> spp.</th>
<th><em>L. monocytogenes</em></th>
<th>RS clostridia</th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw materials</td>
<td>$5.0 \times 10^{14}$</td>
<td>100</td>
<td>P-A$^5$</td>
<td>P-A$^5$</td>
<td>--</td>
</tr>
<tr>
<td>Additives</td>
<td>$1.0 \times 10^{1}$</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Spices</td>
<td>$1.0 \times 10^{15}$</td>
<td>$10^4$</td>
<td>--</td>
<td>$5.0 \times 10^3$</td>
<td>--</td>
</tr>
<tr>
<td>Batters</td>
<td>$5.0 \times 10^{16}$</td>
<td>$10^6$</td>
<td>P-A</td>
<td>P-A</td>
<td>P-A$^b$</td>
</tr>
<tr>
<td>Final products</td>
<td>$5.0 \times 10^{16}$</td>
<td>10</td>
<td>P-A</td>
<td>P-A</td>
<td>P-A$^b$</td>
</tr>
<tr>
<td>Water</td>
<td>10/100</td>
<td>P-A</td>
<td>--</td>
<td>--</td>
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<tr>
<td>Personell</td>
<td>100</td>
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<tr>
<td>Equipment</td>
<td>100/1000</td>
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</table>

$^a$Numbers refer to the applied critical limit.

$^b$Presence–absence at 25 g of sample.

$^c$For plant C the critical limit was $1.0 \times 10^3$ g.

$^d$For plant C the critical limit was $1.0 \times 10^4$ g.

$^e$For plant C the critical limit was $1.5 \times 10^4$ g.

$^f$For plant C the critical limit was $1.0 \times 10^6$ g.

$^g$For plant C the critical limit was $1.0 \times 10^5$ g.

$^h$Presence–absence at 1 g of sample, this parameter was included only in plant’s C HACCP plan.

$^i$< 10 cfu/ml (22 °C), < 100 cfu/ml (37 °C).

$^j$Presence–absence at 100 ml of sample.

$^k$<100/cm² into the slicing and packaging department, <1000/cm² all the other processing surfaces and equipment.

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![Fig. 3](image-url)
oxide and irradiation. Because of the small quantities used, spices do not generally contribute greatly to spoilage flora of the comminuate, although it is possible to contain pathogenic organisms which are heat resistant. The presence of spoilage microflora and pathogens it is not a practical problem if the treatment of meat products such as post-cooking cooling and subsequent storage, are adequate (Brown, 1982).

Microbiological quality of the water was investigated by the examination of 10 samples from each plant. All the samples were shown compliance with the GHP requirements (Table 1).

3.2. Batters and final products

A total of 56 samples were examined from the cutter before stuffing. A low percentage of them found to be compliant (Fig. 5a). Plants A, B and C showed deviation at a percentage of 94%, 88% and 28%, respectively. Fig. 5b shows the combination of the examined microbiological parameters, which cause this deviation. This fact practically indicates that all batters must be handled properly to prevent any potential contamination of final products as well as the areas in which they are processed. Characteristic example is plant C because, although it is using compliant raw materials according to GHP the produced batters showed variation at 28%. This variation possibly is occurred due to: (i) inadequate handling of raw materials during defrozing, deboning or transporting to the next processing step, (ii) cross-contamination during processing, (iii) breaking the chill chain, (iv) long processing and waiting times from one step to another during procedure, (v) faults in hygiene concerning the working staff and the equipment, (vi) resuscitation of microbial counts during defrozing of raw materials.

For the examination of all final products these were divided in three categories: (i) cooked sausages, (ii) cooked cured meat products, (iii) fermented dry sausages. Final products were examined after their heat processing and packaging, except of the fermented sausages examined after their ripening and packaging stage. Eighty-five samples of the final products derived from the same runs of the above mentioned batters were examined and showed compliance in plants A, B and C at a percentage of 87%, 100% and 50%, respectively (Fig. 6a). Deviation came up only from plants A and C at a percentage of 13% and 50%, respectively. Fig. 6b shows the allocation of this deviation among the plants due to the examined counts.

Analysis of variance of the data obtained from TVC and Enterobacteria count shows: (i) a significant difference among the different CCP and GHCP at $P < 0.001$ and $P = 0.008$, respectively (ii) among the plants audited the differences in TVC and enterobacteria were significant at $P = 0.222$ and $P = 0.592$, respectively. The
Fig. 5. (a) Compliance of samples of batters (CCP) and (b) percentage contribution of microbiological variables for the non-compliant samples of batters.

Fig. 6. (a) Compliance of samples of final products (CCP) and (b) percentage contribution of microbiological variables for the non-compliant samples of final products.
interaction of the CCP and GHCP with the plants gave a significant difference $P = 0.008$ and $P = 0.742$ for TVC and Enterobacteria counts, respectively. Figs. 7–10 show the mean averages ± SD (standard deviation) of the microbial populations among the selected CCPs, GHCPs and plants. When the microbial populations were considered between the plants, it is evident that the overall microbial flora was at the same practically level (Figs. 7 and 8). The total microbial flora shows a less marked fluctuation between the CCPs and GHCPs. However, a high population was noted in samples obtained from CCP 3 refer to the preparation of batter in the cutter (Fig. 9). It is evident also that Enterobacteria were at the lowest level in the final product but in the batter were at the highest level (Fig. 10).

Microbiological analysis of all samples revealed the absence of the examined pathogens. Additionally, analysis of the samples showed that the high microbial counts noted in batters were eliminated during thermal treatment. These results indicated that the thermal treatment programs applied for cooked products by the audited plants are effective in assuring their safety. Although thermal treatment is important for the production of safe cooked meat products that have a sufficient shelf life, some other practices are also important. Some of them follow thermal treatment and include slicing, packaging and storage conditions (Tompkin, 1990). As was mentioned above from the examination of samples obtained from plant C demonstrated 50% deviation. This deviation was occurred mainly from the environment of the packaging area during slicing and packaging procedures. The products were mainly contaminated
with lactic acid bacteria (LAB). LAB make up a major part of microbiological flora in the environment of meat plants (Quintavalla, Scaramuzzza, Mutti, Pedrielli, & Barbuti, 1998). Dispersion of LAB may occur from the slicing and packaging machines whenever fermented dry sausages have been sliced or packaged there before (Makela, 1992). So, in plant C the results of our external audit showed that the non-compliance (50%) of the final products was occurred due to the contamination of the processing environment after heat treatment. The same products showed to be compliant with the implemented, in plant C, HACCP system after their heat processing and before slicing and packaging procedures. This finding demonstrates, as we have mentioned before, that an effective GHP is of the outmost importance. The contamination of the products with spoilage and pathogenic bacteria is significantly increased when, the cleaning and sanitizing programs are inefficient and the clean areas of the plant are not well defined.

3.3. Personnel and equipment sanitation

The degree of contamination of the personnel and the surfaces in the processing plants constitutes an important risk factor and should be controlled. Five employees, mainly from the packaging department, and 10 surfaces such as cutter’s, tumbler’s, etc. were examined for each plant, respectively. The results demonstrated that in plants B and C is applied a satisfactory program of cleaning and sanitizing too. However, deviations mentioned in plant A, 16% of the investigated surfaces and a 40% of the examined employees were found to be non-compliant with the GHP requirements. This was due to the inadequate performed cleaning and sanitation program and additionally due to the insufficient continuous training and education of the personnel.

If control is lost because of lack of GHP, product contamination might be broad with all the consequences. Special attention must be paid in this area and the packaging equipment must be cleaned regularly and carefully. They must remain free of meat remnants and be sanitized and kept dry (Price, 1995). Microorganisms, such as L. monocytogenes, in the presence of food remnants, quickly form biofilms resistant to cleaners and sanitizers, and can become a major source of further cross contamination (Genigeorgis, 1996). Moreover, although modern techniques of slicing and packaging are, to a high degree, automated, the cases where the handling of the products by workers are not rare.

4. Conclusion

The adoption of GHP and HACCP preventive strategy to food safety by adapting the existing hygienic control measures to these has been currently applied in Greece by meat industry. This study reveals information on the preliminary application of these safety assurance programmes. Major improvement should be towards the standardization of the raw material specifications, handling of the products and training of the processing personnel. This preliminary application, followed by external audit was the basis for their reviewing and modification.

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