

APPLICATION OF HACCP SYSTEM DURING MEAT SLICES PREPARATION

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Abstract

Each step of preparing meat slices from frozen meat blocks was studied in detail using physicochemical and microbiological tests to identify and eliminate the CCPs which could lead to the hazard occurring. Results showed (a) no significance differences in pH, significance increase at 5% level in surface and center temperature and SPC count of meat after receiving, thawing, cutting and slicing frozen meat blocks were observed (b) Little numbers of psychrotrophic, *Enterobacteriaceae* and *E. coli* were isolated from the surface and center of meat. Meanwhile *Staphylococcus aureus* did not detect after receiving and during processing of meat into slices, and (c) SPC, *Enterobacteriaceae*, and *E. coli* were detected on surfaces of tables and stands of thawing operation, surfaces of cutting boards and tables, surfaces of slicing tables and worker hands. In other hand the surfaces of knives, carriers, and polyethylene sheets were free from the microorganisms.

Keywords: HACCP, psychrophilic, standard plate count, good manufacturing practice

ET DOĞRAMA İŞLEMİ SIRASINDA HACCP SİSTEMİNİN UYGULANMASI

Özet

Dondurulmuş gövde etin doğranmasındaki her aşama, tehlike oluşturabilecek kritik kontrol noktalarının belirlenmesi ve elemine edilmesi amacıyla fizikokimyasal ve mikrobiyolojik analizlerle araştırılmıştır. Sonuçlara göre; (a) pH'da önemli bir değişiklik olmamıştır, merkez ve yüzey sıcaklığı ile toplam aerobik mezofilik bakteri sayısında etin alınması, çözülmesi, kesilmesi ve doğranması aşamalarında %5 düzeyinde önemli fark görülmüştür (b) Et yüzeyi ve merkezinden az sayıda psikrotrofik bakteri, *Enterobacteriaceae* ve *E. coli* izole edilmiş ancak hiç bir aşamada *Staphylococcus aureus* görülmemiştir (c) Et çözme masalarında ve askılarında, kesme tahta ve masalarında ve işçilerin ellerinde toplam aerobik mezofilik bakteri, *Enterobacteriaceae* ve *E. coli* görülmüştür. Diğer taraftan, bıçak yüzeylerinde, taşıyıcılar ve polietilen levhalarda bu mikroorganizmalara rastlanmamıştır.

Anahtar kelimeler: HACCP, psikrofilik, toplam aerobik mezofilik bakteri, doğru üretim uygulamaları

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INTRODUCTION

It is necessary to identify the level of risks involves during preparation and consumption meat, poultry and fish products due to use improper heating and cooling conditions such as an inadequate cooking, improper warm holding, preparation too far in advance of serving, storage at ambient temperature. This can be done by applying Hazard Analysis Critical Control Points (HACCP) system to define the Critical Control Points (CCPs) and their limits, exercised control which should used to prevent and eliminate hazards in addition to apply the criteria for monitoring CCPs and corrective actions should be taken (1).

In this study, the main aim is to apply the HACCP system during producing meat slices from frozen meat blocks in meat processing unit to use in preparing Escalope paneé sandwich in series of restaurant branches of one of the large and famous fast food company at Alexandria City, Egypt. Each steps of this food operation was subjected for physicochemical and microbiological tests to identify the factors lead to hazard occurring. The HACCP team was formed from the manager of quality control and representatives from the production and maintenance departments.

MATERIALS AND METHODS

Materials

Samples from five different batches of Brazilian imported back rip boneless beef meat blocks with nine months shelf life at -18 °C were collected after receiving and during processing into slices inside the meat preparation unit as explained in Fig 1. The different collected samples were packed in polyethylene bags and kept in ice boxes during transporting to the laboratory for analysis. Also swabs from surface of tables, utensils, equipments, and hand workers were taken to determine the efficiency of sanitation program applying in such unit.

Methods

Microbiological methods: Swabs of 100 cm² of each meat samples, tables and stands as well as those of workers hands were mixed with the 100 ml of sterilized peptone water for 5 min.

Receiving of frozen meat at 10 °C (Storage at -15 °C) ⇒ Thawing at room temperature (22±3 °C) and 79% relative humidity (RH) for 4-5 hrs. ⇒ Dressing by removing surrounded fat layer ⇒ Cutting into thick portion with 5.0-7.0 cm thickness. ⇒ Slicing into slices with 0.5-0.8 cm thickness. ⇒ Knocking to reduced thickness to 2-3 mm with 40-50 g weight with 8-9 cm length and 5-6 cm wide. ⇒ Wrapping in large polyethylene sheet. ⇒ Packing in polystyrene boxes with 10kg capacity. ⇒ Storing at 4 °C ⇒ Transporting by refrigerated vehicle at 1-5 °C to restaurant branches.

Fig 1. Outline of meat slices preparation.

Appropriate dilution from 10¹ to 10⁶ were prepared to select the suitable one for enumeration using standard microbiological pour plate technique and the recommended culture media described by the International Commission on Microbiological Specification for Food (2). Plate count agar medium was used to enumerating the standard plate count (SPC) and psychrotrophic (PPC) bacteria after incubating the plates at 35-37 °C for 48 hrs and 7 °C for 10 days respectively. Violet red bile agar with methyl umbeliferyl glucuronide (VRBMUG) selective media was used for isolation of coliform, Gram negative enteric bacteria and rapid detection of *E. coli* after incubating the plates at 37 °C for 18-24 hrs. Colonies of lactose negative *Enterobacteriaceae* are colorless and those of lactose positive are red often surround by a forbid zone due to precipitation of bile acids. Light blue fluorescent colonies under 336 nm Ultraviolet light (Merck, Germany) denote as *E. coli*. Baird Parker agar medium was used to detect *Staphylococcus aureus* after incubating the plates at 35-37 °C for 48hrs. The growth black shiny colonies with narrow white margin and surrounded by clear zones were counted as *Staphylococcus aureus*. TA latex slide agglutination test (Oxoid) was used to differentiation between coagulase positive and negative Staphylococci (3).

Physicochemical methods: The temperature of the surface and center of meat (8-10 cm depth from the meat surface) was measured using Comark Thermocouples Testo, Germany. pH value was measured after blending meat samples with distilled water at a 1:10 w/v ratio in a blender using pH meter Testo, Germany, at room temperature (22±3 °C) (4).

Statistical methods: A simple complete randomized design was used (5) to study the influence of five received frozen meat block batches, their preparation operation into slices and the interaction between the previous two factors on the quality of the resulted meat slice

RESULTS AND DISCUSSIONS

The results of monitoring the influence of each step of meat slices preparation on the presence of hazards were as following.

Receiving

Temperature and pH

The temperature of the received five batches of meat was approximately varied from -8.1 to -1.5 °C with an average of -4.8 °C on the surface, and from -12.2 to -3.2 °C with an average of -7.7 °C on the center. There was a significant difference at a 5% level in both surface and center temperature among the five received frozen meat batches. Also there is a parallel relationship between the surface and center temperature of the received frozen meat batches. Batch two had the lowest surface (-8.1 °C) and center (-12.2 °C) temperatures while the highest ones were observed on the surface of batch three (1.5 °C) and center of batch three and four (~-3 °C). These variations indicted that inadequate temperatures were used either during storage and/or transporting of frozen meat. The recommended storage temperature for frozen food product is below -18 °C (6). At such temperature, microbial growth is completely stopped in addition to both enzymatic and non-enzymatic reaction are continued at much slow rate during storage of frozen product. It was mentioned in the Egyptian standards (7) that frozen meat should be stored and/or transported in refrigerated vehicles at -18 °C. The rules of the meat processing unit allow delivering frozen meat at a temperature not more than -10 °C and also allowed to transport frozen meat in refrigerated vehicles at a temperature not more than 10 °C. The storing and transporting of frozen meat below -10 °C stopped microbial growth and slowed the enzyme action (8). According to the above results, control of the frozen meat receiving temperature is essential to avoid the deterioration of its

quality particularly the uncontrolled multiplication of microorganisms.

The pH of the surface and center of the five received meat batches varied from 5.82 to 5.85 with an average of 5.84 and from 5.79 to 5.85 with an average 5.81, respectively. It is known that after rigor period, the pH of meat is increased to 5.9 - 6.2. Both pH and temperature impact the physical properties and proteolytic enzyme activity of meat (9). The inspection of the physical state, surface colour and cleanliness, presence of abnormalities extraneous materials, contamination by pests, integrity of any wrapping or packaging, internal temperature, microbial load, pH, in addition to the temperature and hygienic conditions of frozen meat are usually done during receiving step (8).

Microbiological quality

Most of the microorganisms that cause spoilage of meat are either present at the time of slaughter or are introduced during dressing, cooling and cutting in the processing room. Results in Table 1 showed that the total count of SPC on the surface and center of the five received frozen meat batches were less than 6 log cfu/ cm², the acceptable limit stated in the Egyptian frozen meat standard (7). May (10) stated that meat having an aerobic plate count of less than 5 log cfu/ cm² have an acceptable quality, while those having 7 to 8 log cfu/ cm² are generally have detectible off flavour. Bacterial numbers above 8 log cfu/ cm² result in some structural changes particularly slime formation.

Data in Table 2 postulated that both the surfaces and centers of the five received frozen meat batches contained little count of psychrotrophic bacteria. However these low numbers, significant differences were noticed in the count of such bacteria among the five frozen meat batches. The term psychrotrophic was suggested to classify microorganisms capable of multiplying at 5 °C and below (11).

Results in Table 3 indicated that both surface and center of the five received frozen meat batches contained few numbers of *Enterobacteriaceae*. Also, *E. coli* was only detected in low count, 2.48 log cfu/ cm², on the meat surface of the batch four. *S. aureus* was completely absent from the

Table 1. SPC count (log cfu/cm²) of frozen meat blocks.

Process	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5	
	Surface	Center	Surface	Center	Surface	Center	Surface	Center	Surface	Center
Receiving	2.57	2.49	2.54	2.48	3.58	2.52	3.60	2.49	3.64	2.48
Thawing	3.71	2.60	3.74	2.48	4.70	3.56	3.66	2.52	3.81	2.61
Cutting	4.57	2.63	4.71	2.56	4.73	2.56	3.86	2.54	3.83	2.68
Slicing	4.70	-	4.95	-	4.77	-	4.88	-	4.93	-

Significant variation at 0.05 % level, LSD for process, batch and interaction: - 0.1449, 0.1620, 0.3240, respectively on surface but it was 0.0292, 0.0377, 0.0653 in center.

Table 2. Psychotropic count (log cfu/cm²) of frozen meat blocks.

Process	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5	
	Surface	Center	Surface	Center	Surface	Center	Surface	Center	Surface	Center
Receiving	N.D	N.D	N.D	N.D	N.D	N.D	2.68	2.48	2.52	2.48
Thawing	N.D	N.D	N.D	N.D	2.64	N.D	2.74	2.52	2.72	2.49
Cutting	N.D	N.D	N.D	N.D	2.77	N.D	3.36	2.60	2.75	2.54
Slicing	N.D	-	2.68	-	2.91	-	3.48	-	3.56	-

Significant variation at 0.05% level, N.D = Not Detected. LSD for process, batch and interaction: - 0.1987, 0.2222, 0.4444, respectively on surface but it was: - 0.1719, 0.2220, 0.3845 in center.

surface and center of the five received batches of frozen meat. Generally frozen food deteriorated by psychrotrophic, mesophilic and thermophilic microorganisms when are subjected to temperature extremes and freezing/thawing cycles (11). Fecal microorganisms were very sensitive for freezing storage while psychrotrophic was more resistance for this affect (12). Staphylococci can be easily destroyed at freezing conditions while their enterotoxins can survive in all food processing operations (13). The above results indicated that the variations in the recorded temperature of the received frozen meat batches did not affect their physical properties and microbiological quality.

Thawing

Temperature and pH

Data revealed that thawing process of frozen meat was stopped when its surface and center temperatures ranged from -1.7 to 4.5 °C and -5 to -2.8 °C, respectively. The rules of the meat processing unit allow thawing of frozen meat to a temperature ranges from 1.0 to 5.0 °C on the surface. There were significant differences in both surface and center temperatures of frozen meat among the five studied batches. These variations were principally attributed to differences in the initial temperatures of frozen meat before thawing in addition to the variation in the room

Table 3. Enterobacteriaceae count (log cfu/cm²) of frozen meat blocks.

Process	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5	
	Surface	Center	Surface	Center	Surface	Center	Surface	Center	Surface	Center
Receiving	2.36	2.48	2.48	N.D	2.62	2.53	2.54	2.48	2.60	2.48
Thawing	3.54	2.49	3.71	2.59	2.82	2.73	2.80	2.53	4.56	3.63
Cutting	3.56	2.48	3.56	2.52	3.72	2.51	3.73	2.48	3.92	2.53
Slicing	3.85	-	4.85	-	3.72	-	3.67	-	4.49	-

Significant variation at 0.05% level, N.D = Not Detected, LSD for process, batch and interaction: - 0.2260, 0.2527, 0.5054, respectively on surface but it was 0.0875, 0.1129, 0.1956 in center.

temperature at which thawing process was carried out (22 ± 3 °C). According to the average temperatures of the five thawed batches, the thawing process was ended when the temperature of the surface and center reached to -4 and 1 °C, respectively. At such temperatures the meat can be easily cut and sliced manually. During thawing at room temperature, the surface of the frozen meat was first subjected to air of the room atmosphere and thawed first. Generally frozen products should be thawed slowly at temperature just above freezing (11).

The pH of the surface and center of the five thawed frozen meat batches varied from 5.82 to 6.07 with an average of 5.91 and from 5.81 to 5.90 with an average of 5.83 respectively. Thawing process caused a significant rise in pH of meat. This rise was increased from 5.84 to 5.91 and from 5.81 to 5.83 in surface and center of meat after thawing respectively. However such variations, the pH of meats was near from the ultimate pH (5.6) and far from the pH (6.2) which causing the dark, firm and dry meat (14).

Microbiological quality

Data in Table 1 indicated that the SPC of meat was significantly increased in both surface and center of meat after thawing process. Also significant differences were noticed in this count among the five studied batches of meats. Such differences can be attributed to many factors such as the fluctuate in room temperature during thawing, initial temperature of frozen meat before thawing, the aeration condition and relative humidity around frozen meat during thawing (such conditions affect the defrosting time), the cleaning and sanitation of the thawing shelves and stands, meat packages etc. However such increase, the count of SPC was very far from the 10^6 stated in Egyptian standards to indicate the spoilage of meat. According to Soto *et al.* (15) the condensation of moisture on the surface of the meat should be avoided during thawing. This may cause speeding up of microbial growth. Also, the air temperature should not exceed 20 °C if thawing is conducted in still air to prevent the warm up of meat surface and the growth of microorganisms on the surface before the center. As shown in Table 2 the psychrotrophic bacteria were detected only on the surface of the three

and in the center of the two thawed meat batches. The load of these bacteria found in low count especially in center of thawed meat. Generally, slowly thawing of frozen food allows growing the psychrotrophic flora and slowing down the multiplication of any present pathogens until thawing is complete (15).

The load of the *Enterobacteriaceae* was significantly increased on both surface and center of frozen meat after thawing process, Table 3. This may be due to the growth of these bacteria and from the outside contamination of meat during thawing. Generally the mean count of *Enterobacteriaceae* was 3.48 log cfu/ cm² and 2.60 log cfu/ cm² on the surface and center of thawed meat, respectively. *E. coli* was absent from the center and detected only on the surface of two batches, 4 and 5, of the thawed frozen meat at a count less than 2.11 log cfu/ cm². The count of such bacteria on the surface was nearly stable after thawing the frozen meat of batch four and detected after thawing frozen meat of batch five. This is an indication that the contamination source of these bacteria was mainly from employee hands, meat handling tools and utensil used during thawing. The low resistance of *E. coli* to low temperature was behind their inability to multiple in numbers during this process. *Staphylococcus aureus* did not detect in both surface and center of the thawed frozen meat. Soto *et al.* (15) showed that defrosting methods had no effect on the counts of aerobic mesophilic bacteria, Coliforms, streptococcus spp. and sulphite reducing clostridium. Also, fecal Coliforms are very sensitive for frozen storage. Generally thawing and processing the meat (such as cutting, mincing ...) at temperatures between -5 °C and -2 °C under hygienic conditions are important in lowering the growth of microorganisms. The swabs taken from worker hands, the received meat table and thawing stands indicated the presence of SPC, *Enterobacteriaceae* and *E. coli*. The count of such bacteria was low. The SPC count was varied from not detected to 2.54 log cfu/ cm² on table surface, not detected to 2.58 log cfu/ hand on worker hands, and 2.48 to 3.65 log cfu/ cm² on the surface of frozen meat thawing stands. The load of both *Enterobacteriaceae* and *E. coli* were ranged from not detected to 3.64 and to 2.52 log cfu/ cm² respectively, only on the surface of table and surface of thawing stands.

Cutting

During this process, the undesired tissues are removed.

Temperature and pH

Because, the cutting of the meat masses was carried out after thawing and when the surface and center temperatures of thawed meat were ~ 0.8 and -4 °C, respectively. The loss rate of water holding capacity (WHC), toughness of the texture, drip formation was reduced. Also, this helps in cutting meat masses into pieces. The surface and center temperatures of the thawed cutted meat pieces were differed from -0.53 to 4.7 °C with an average of 2.3 °C and from -2.6 to 1.7 °C with an average of -0.9 °C, respectively. Generally, the meat batches having high temperature during receiving and thawing also gave pieces with high temperature.

It was found that the pH values of surface and center of cut meat pieces ranged from 5.82 to 6.19 with an average of 5.95 and from 5.82 to 5.91 with an average of 5.87 respectively. Only the meat pieces of the first frozen batches had significant higher surface pH value (6.91) than other's batches. Generally, the rate of the changes in pH value during cutting was relatively low due to the low temperature of the meat which lowered from the biochemical changes and growth of microorganisms.

Microbiological quality

Data in Table 1 showed that the count of SPC was increased after cutting process on both the surface and center of the resulted meat pieces. Generally these levels were far from the $6 \log \text{cfu}/\text{cm}^2$ indicated spoilage meat as stated in the Egyptian frozen meat standards. The low temperature of meat masses, following the sanitation practices, and the rapid application of cutting operation were behind the low load of SPC in cutted meat pieces (16). Hinton *et al.* (17) stated that defrosted meat seems to spoil faster than chilled one because of the damage to tissues. Also, the exudates formed during thawing and cutting enhances microbial growth. The good quality meat has aerobic plate count at 25 °C under $5 \log \text{cfu}/\text{cm}^2$ (17). The results in Table 2 showed that psychrotrophic bacteria did not detect in the surface and center of the cutted pieces from the

received frozen meat batch – one and two. The same observation was noticed also for the center of cutted meat of batch three. Numbers of psychrotrophic bacteria cells on meat surface can be as low as $2 \log \text{cfu}/\text{cm}^2$ (17).

As reported in Table 3, *Enterobacteriaceae* was detected in both surface and center of cutted meat pieces. Cutting operation caused a significant reduction in the count of such bacteria on the center of meat pieces. These bacteria may be removed with the resulted drip during thawing process and with an exudates forming during cutting. Also, no significant differences in the mean count of such bacteria on the surface of meat were observed before and after cutting, this indication that the cutting process carried out under sanitation condition.

E. coli did not detect in the center and fluctuated from not detected to $2.54 \log \text{cfu}/\text{cm}^2$ on the surface of the cutted meat pieces from the received five frozen batches. This is an indication that the contamination sources of these bacteria may due to the cutting knives, hand of employee's and surface of cutting tables. In other hands, *Staphylococcus aureus* did not detected in the surface and center of the cutted meat pieces. Hinton *et al.* (17) showed that at a temperature below 4 °C, most pathogenic bacteria fail to grow. Swabs' from knives, cutting board and tables surface in addition to hands of employees indicated the presence of the following microorganisms; SPC, *Enterobacteriaceae* and *Staphylococcus aureus* on cutting board surface, SPC, *Enterobacteriaceae* and *E. coli* on cutting table's surface.

Slicing

The aim of this process is to cut the resulted meat pieces into thin slices having nearly the same sizes and dimensions.

Temperature and pH

The temperature of the resulted meat slices was ranged from -1.1 to 6.2 °C with an average of 3.2 °C. This means that these slices were still chilled or cooled. At temperature 7 °C no growth was noticed by Hinton *et al.* (17) for *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* spp and *Clostridium* spp.

No significance differences were noticed in the average of pH value of meat after slicing process. This was due to the application of cutting and slicing processes in short time at low temperature which sequentially reduced the rate of biological changes especially acid-base equilibrium (pH) one (16).

Microbiological quality: As shown from Table 1 the highest count of SPC was found in meat after slicing. No significance differences were noticed in this count among the slices resulting from the received five frozen meat batches. These values were far from the load reported to indicate the spoilage of meat in Egyptian standards, $6 \log \text{cfu/cm}^2$.

Data in Table 2 showed that psychrotrophic bacteria was detected in meat slices of four of the five received frozen batches, number 2,3,4 and 5. Also, the numbers of *Enterobacteriaceae* were gradually increased after slicing process as illustrated in Table 3. This increase was mainly due to the growth and multiplication of these bacteria through this process. *E. coli* was increased in meat after slicing process to nearly $2.54 \log \text{cfu/cm}^2$ in all batches due to both the multiplication of these bacteria and also from the contamination particularly employee hands. Generally the source of the detected few numbers of *Staphylococcus aureus* in meat slices may comes from meat handling. The results of microbiological examination of swabs of slicing tables, boards, knives, worker hands and polyethylene sheets showed the absence of SPC, *Enterobacteriaceae*, *E. coli*, coliform and *Staphylococcus aureus* from knives and wrapping polyethylene sheets. SPC bacteria were detected on the surfaces of slicing table, cutting boards and worker hands. *Enterobacteriaceae* was also present on the surface of both slicing tables and cutting boards. *E. coli* was found only on the surface of cutting board and coliform was isolated from the employee hands. Such results explain the contamination sources of meat with such microorganisms during processing into slices and also the need to monitor and revise the applying sanitation program in the meat processing unit.

CONCLUSIONS

The control of critical control points fall into two categories: **(I)** prevention of cross contamination microorganisms or foreign matter by processing and employees to product rout by:- **(a)** use of tongs and gloves for handling frozen, thawed, cut and sliced meat., **(b)** washing, rinsing and sanitizing all utensil. **(c)** Regular use of sanitizers for wiping all meat contact surfaces and **(d)** stringent application of hand washing and hand sanitizer. **(II)** prevention of microbiological growth through abuse time and risen in temperature of delivery or receiving, storage, thawing, cutting, slicing and handling of meat by **(a)** defining shelf life and storage conditions of the received frozen meat and ensuring that these are adhered also during transporting and distribution, **(b)** operating to strict first in first out (FIFO) as a main principle on store management, **(c)** defining maximum preparation time and temperature for all processing steps of the slices preparation from frozen meat, and **(d)** auditing of the continuous monitoring of sanitation and hygienic programs in addition to the application of good manufacturing practice (GMP) at the processing unit. It is preferred that all of processing steps are detailed in an operation manual. The monitoring of the CCPs should be checked by estimating temperature, time, pH and microbiological analysis using rapid tests and their results should be documented and recorded in suitable format, validated and signed by the responsible persons as well as kept for at least one year.

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