OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

The Effect of Time and Temperature Variations on the Microbial Load and Deterioration Criteria of Leftover Cheeseburger Sandwiches

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Abstract | Sixty cheeseburger sandwiches were bought from sixty different fast food outlets and street vendors in Cairo as well as the governorate of Giza. Sandwiches were divided into three groups; room temperature (25°C), refrigerator (5°C), and high temperature (37°C). The three groups were examined after 30, 60, and 120 minutes for bacteriological quality (aerobic plate count, psychrotrophic, total coliform, fecal coliform, *E.coli*, total staphylococci, and salmonella) count and deterioration criteria (pH, TVBN, and TBA). The findings showed significant growth in aerobic plate count, psychrotrophic, total coliform and staphylococci count while salmonella failed to be detected under different storage temperatures. Moreover, *Citrobacter diversus*, *Citrobacter freundii*, *Serratia fonticola*, *Enterobacter intermedius*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella oxytoca*, and *E.coli* can be isolated from sandwiches kept under high temperature (37 °C) for 120 minutes. The deterioration criteria tests discovered that the increase of storage temperature as well as period of storage resulted in a significant raise in pH, TVBN, and TBA values. Moreover, deterioration criteria of examined leftover sandwiches kept under high temperatures (37 °C) for the longest period (120 minutes) exceeded the permissible limit according to E.S.S. (2005). According to this, holding sandwiches at high temperature for an extended period increased microbial load and deterioration criteria resulting in food-borne diseases and health risks and consequently rendering them unfit for human consumption.

Keywords | Cheeseburger, Bacteriological, Leftover, TVBN, TBA

Received | August 12, 2021; **Accepted** | August 18, 2021; **Published** | September 25, 2021
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Citation | Malak NML, Soliman NSM (2021). The effect of time and temperature variations on the microbial load and deterioration criteria of leftover cheeseburger sandwiches. Adv. Anim. Vet. Sci. 9(11): 1925-1932.
DOI | http://dx.doi.org/10.17582/journal.aavs/2021/9.11.1925.1932
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

As a result of the hectic lifestyle and most people, especially youth spend a long time outside their homes, so this gives the chance for the fast food industry to grow up all over the world. Globally, there are approximately 2.5 billion persons eat fast food daily (FAO, 2007). Ready to eat foods (RTE), such as hot dogs, fermented sausage, burger, shawarma, and meatballs, are products that are sold at the quick-service restaurants and can be consumed immediately without further preparation (Tsang, 2002). Among these different types of fast foods, Sandwich is the most popular way of consumption because of its easy and quick preparation, delicious taste beside it contains different types of foods as producers add salads from fresh vegetables to sandwiches as cabbage, carrot, cucumber, onion, ketchup, and mayonnaise. However, the quality of these sandwiches greatly depends on several factors such as the initial load of microbial contamination of meat and raw vegetables, method of preparation, time, and temperature

of storage from the time of preparation till consumption (NRC, 1985).

Despite of the above-mentioned advantages of RTE sandwiches, many studies have been proved that the higher consumption of RTE products increases the danger of foodborne infection and intoxication as a result of different sources of contamination for these street foods (Gilbreth et al., 2005; Tambekar et al., 2008; De Vogli et al., 2014). Food Agriculture and organization (FAO) reported that, approximately 1.3 billion tons of food are exposed to spoilage worldwide annually from the time of processing till consumption (FAO, 2011). Every year, almost thirty percent of people in developing countries suffering from a foodborne illness, and at least two million people died from the diarrheal disease worldwide in 2000 (WHO, 2002). Globally, Salmonella and *Staphylococcus aureus* are the most causative pathogens of foodborne infection and intoxication while infection with salmonella and *E. coli* O_{157} resulting in hospitalization but salmonella and *Listeria monocytogenes* may lead to death (CDC, 2011). Moreover, the presence of *E.coli, Staphylococcus aureus* and salmonella in foods is an indicator that food handlers ignore the lowest level of proper hygienic practices (Lues et al., 2006). *E.coli* is a well-known food-borne pathogen that caused several disease outbreaks (Scotter et al., 2000). In addition, *Staphylococcus aureus* has been informed in severe cases of diarrhea and, as reported the principal cause of food poisoning gastroenteritis among consumers (Davies and Board, 1998).

The dangers of food poisoning from these fast food increases by storing them at room temperature for a long time as meat is high perishable food as it is rich in protein, fat, and water. Also, pathogenic bacteria grow faster in meat due to high pH and water activity (Dave and Ghaly, 2011). So, if these products not properly handled, stored, and preserved they will be exposed to the growth of pathogenic bacteria and resulted in public health hazards (Fratianni et al., 2010). Furthermore, improper processing and storage conditions will cause breakdown of proteins and lipids found in meat and meat products (Dave and Ghaly, 2011) causing abnormal odor, color, consistency, and taste of prepared food (Borch et al., 1996).

Many people leave leftover foods at room temperature or in the refrigerator for a long time, then they eat them with or without reheating which resulted in foodborne illness due to the growth of mesophilic and psychrotrophic bacteria. Leftovers are foods that remain uneaten after a meal (Merriam-Webster, 2021). These leftovers if kept at higher room temperature for a long time resulted in fast food deterioration. So, putting away leftovers should be in conditions that keep them safe for the longest period till their consumption in a way that retard the enzymatic processes and retard microbial growth. Consequently, the goal of this current research is to evaluate the changes in microbiological and deterioration criteria of cheeseburger sandwiches sold in Cairo and Giza governorate fast food restaurants under various conditions held at high temperature (37 °C) as in summer season as well as at room temperature as in winter season (25 °C), and at low temperature (refrigerator 5 °C) in order to determine the best appropriate method for holding the leftover sandwiches with minimal health hazards on consumers to ensure the safety and quality of such foods.

MATERIALS AND METHODS

Collection of Samples

Sixty samples of cheeseburger sandwiches, were obtained from various locations in Cairo and the Giza governorate, and aseptically transferred, in an insulated icebox to the laboratory. The ingredients of all sandwiches were beef burger, cheese, and vegetables such as tomato, cabbage, lettuce, and cucumber. Sandwiches were divided into three groups; the first, the second, and the third group was held at room temperature (25°C), in a fridge at 5°C, and the third at temperature 37°C respectively. All these groups were subjected to bacteriological examination and deterioration criteria after 30, 60, and 120 minutes. Samples were taken in equal portions from their ingredients (beef, cheese, and vegetables) for examination of bacteriological quality and deterioration criteria.

Bacteriological Examination

Ten grams of all ingredients of cheeseburger sandwiches were aseptically added to 90 ml ringer's solution to make decimal dilutions according to APHA (2001). On standard plate count agar, inoculate double plates with 100µ of previously prepared decimal dilutions for enumeration of aerobic plate count (APC) as described in ISO, 2005, psychrotrophic count according to ISO (2002), on Paired Barker agar media for total staphylococci count (APHA, 2001). Additionally, isolation and identification of coliforms were examined in sandwiches based on BAM (2013), as well as serological identification of isolated *E.coli* strains was carried out in accordance with Kok et al. (1996). Also, isolation of salmonella was done as described by ISO, (2002).

Deterioration criteria

Five grams of all ingredients of cheeseburger sandwiches were mixed with twenty ml distilled water for 10–15 seconds and insert the probe of digital pH meter to measure the pH for samples (Kandeepan et al., 2009). On the other hand, five grams of sandwiches were added to 300 ml distilled water, and two grams of magnesium oxide for the micro distillation method which was used to determine the total volatile base nitrogen (TVBN; mg percent sample) as

done by Kearsley et al. (1983). Also, five grams were added to 15 ml of distilled water and homogenized with a stomacher then filtered in order to determine the thiobarbituric acid (TBA, mg malondialdehyde /kg) value of samples, Take one ml of the filtrate to 1 ml of TBA reagent, one ml of trichloroacetic acid, and fifteen µ of Butylated hydroxytoluene (BHT) then the tubes were taken in boiling water bath for 15 minutes then make cooling, centrifugation, reading absorbance at 531 nm using spectrophotometer (Du and Ahn's, 2002).

Statistical analysis of results

SPSS 17.0 was used to analyze the statistical results for the three separate replicates (SPSS Inc, Chicago, IL, USA). Means differences of bacteriological testing, pH, TVBN, and TBA values between various periods and temperatures were measured by the use of one-way analysis of variance (ANOVA) as well as multiple comparisons of means were carried out by the Post Hoc (least square difference test, LSD). At the ($P<0.05$), differences were found significant.

RESULTS AND DISCUSSION

Effect of changes in times and temperatures on the bacterial count (mean log_{10} CFU/g) of the leftover cheeseburger sandwiches

The primary goal of microbiological examinations is to guarantee the safety of prepared sandwiches for consumers as well as to ensure that sandwiches are made from high-quality raw materials have not deteriorated or are contaminated during preparation, handling, storage, and marketing. Aerobic plate count (APC) is one of the most collective used tools used to explore the safety of food during the processing, preparation, and handling (ICMSF, 1978; Jinadasa, 2010). The APC of leftover cheeseburger sandwiches held at different storage times and temperatures are presented in (Table 1), with a significant ($P < 0.05$) increase by increasing storage temperature, but no significant ($P > 0.05$) change between samples kept at the same temperature by increasing the period, but there was a significant ($P < 0.05$) rise in the APC when sandwiches kept at 37 °C for two hours which reach to (9.41 log_{10}CFU/g). The APC of cheeseburger sandwiches just after processing was 5.60 log_{10} CFU/g (Shaltout et al., 2015) while in the beef burger was 5.53 log_{10} CFU/g (Easa, 2010). A High initial count in the analyzed sandwiches may be the result of contaminated raw materials, contamination of sandwiches after cooking and poor storage or handling conditions (Huck et al., 2007). The APC increased by keeping sandwiches at a high temperature at 37 °C for a long time (120 minutes) which agrees with Khater-Dalia et al. (2013) who stated that storage of sandwiches for long time at a high temperature resulted in high microbial count. According to the microbiological guidelines of Ready-To-Eat (Centre for Food Safety, 2007) where foods considered unacceptable if the total aerobic bacterial count exceed 10^6 log_{10}CFU/g so sandwiches stored at room temperature (25 °C) and high temperature (37 °C) after one and two hours considered unsatisfactory from microbiological quality. Moreover, the total psychrotrophic count significantly ($P< 0.05$) increased in sandwiches by raising the temperature and time of storage where the highest count was recorded in leftover sandwiches kept at 37 °C for 2 hours which reach 6.90 (log_{10} CFU/g). The presence of psychrotrophic bacteria on beef burger sandwiches at different storage periods may be due to the growth of Achromobacter and pseudomonas bacteria which able to grow on the surface of meat kept at low temperatures (Petersen and James, 1998).

Total coliform count (log_{10} CFU/g) significantly ($P< 0.05$) increase by keeping cheeseburger sandwiches at different storage temperatures and different storage times. Moreover, sandwiches which left at high temperatures (37 °C) for long period (120 minutes) scored the highest total coliform count which exceeds the permissible limit (>10^4 CFU/g) described by Food Standards (2018) and consequently threatens public health. The results in harmony with Shaltout et al. (2015).

The prevalence (%) of coliforms in the examined cheeseburger sandwiches kept at high temperature (37 °C) for 120 minutes (Figure 1) showed that 15% (9/60) of samples were contaminated with coliform. Furthermore, two isolates from suspected six isolates were *E. coli* O_{25} using slide agglutination test for serological identification. Moreover, Coliform members are considered as indicator organisms for enteric pathogens in food. The occurrence of coliform in ready to eat sandwiches indicates that washing and hygiene processes during food preparation and packing are inadequate (Jay, 2005).

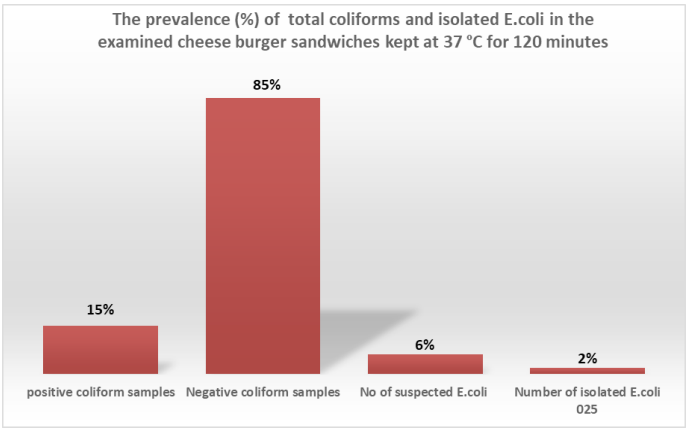

Figure 1: The prevalence (%) of total coliform and isolated *E.coli* in the examined cheeseburger sandwiches kept at 37 °C for 120 minutes

Moreover, the fecal coliform count of examined leftover cheeseburger sandwiches significantly ($P<0.05$) increase by

Table 1: Effect of changes in times and temperatures on the bacterial count (mean $\log_{10}$ CFU/g) of the leftover cheeseburger sandwiches (n=60)

Temperature Time	5 °C	25 °C	37 °C
Aerobic Plate count (APC)			
30 minutes	$4.75^{c,A}\pm0.30$	$5.95^{b,A}\pm0.33$	$6.54^{a,C}\pm0.10$
60 minutes	$5.47^{c,A}\pm0.31$	$7.00^{b,A}\pm0.21$	$7.74^{a,B}\pm0.09$
120 minutes	$6.55^{c,A}\pm0.27$	$8.55^{b,A}\pm0.09$	$9.41^{a,A}\pm0.16$
Total psychrotrophic count			
30 minutes	$2.40^{c,C}\pm0.11$	$2.89^{b,C}\pm0.16$	$3.94^{a,C}\pm0.15$
60 minutes	$2.97^{c,B}\pm0.19$	$3.50^{b,B}\pm0.10$	$5.44^{a,B}\pm0.12$
120 minutes	$3.95^{c,A}\pm0.18$	$4.98^{b,A}\pm0.22$	$6.90^{a,A}\pm0.11$
Total Coliform content "MPN"			
30 minutes	$1.24^{c,C}\pm0.06$	$1.62^{b,C}\pm0.10$	$2.11^{a,C}\pm0.10$
60 minutes	$1.65^{c,B}\pm0.06$	$2.12^{b,B}\pm0.11$	$2.92^{a,B}\pm0.14$
120 minutes	$2.09^{c,A}\pm0.05$	$2.61^{b,A}\pm0.16$	$4.22^{a.A}\pm0.16$
Fecal coliforms "MPN"			
30 minutes	$0.15^{c,B}\pm0.08$	$0.33^{bc,B}\pm0.12$	$1.32^{ab,B}\pm0.28$
60 minutes	$0.32^{c,B}\pm0.18$	$0.60^{bc,B}\pm0.21$	$2.22^{ab,B}\pm0.20$
120 minutes	$0.67^{c,A}\pm0.23$	$1.36^{b,A}\pm0.48$	$3.25^{a,A}\pm0.29$
Total Staphylococci count			
30 minutes	$2.95^{c,C}\pm0.15$	$3.55^{b,C}\pm0.08$	$4.21^{a,C}\pm0.05$
60 minutes	$3.57^{c,B}\pm0.11$	$4.60^{b,B}\pm0.09$	$5.70^{a,B}\pm0.06$
120 minutes	$4.34^{c,A}\pm0.07$	$5.85^{b,A}\pm0.10$	$7.21^{a,A}\pm0.23$
Salmonella			
30 minutes	$<2a,^{A}$	$<2a,^{A}$	$<2a,^{A}$
60 minutes	$<2a,^{A}$	$<2a,^{A}$	$<2a,^{A}$
120 minutes	$<2a,^{A}$	$<2a,^{A}$	$<2a,^{A}$

Data indicates means±SE

$^{a-c}$, $^{A-C}$ Means with different superscripts for temperature and time in each row and column respectively differ significantly at (*P<0.05)*

Table 2: Effect of changes in times and temperatures on deterioration criteria of leftover cheeseburger sandwiches (n=60)

Temperature Time	5 °C	25 °C	37 °C
pH			
30 minutes	$5.21^{c,C}\pm0.06$	$5.96^{b,C}\pm0.13$	$7.18^{a,B}\pm0.26$
60 minutes	$5.87^{c,B}\pm0.12$	$6.94^{b,B}\pm0.23$	$8.10^{a,A}\pm0.21$
120 minutes	$6.21^{c,A}\pm0.13$	$7.46^{b,A}\pm0.23$	$8.60^{a,A}\pm0.23$
Total Volatile Base Nitrogen (TVBN) (mg/100g)			
30 minutes	$10.07^{c,C}\pm0.43$	$11.4^{b,C}\pm0.26$	$14.37^{a,C}\pm0.29$
60 minutes	$12.60^{c,B}\pm0.35$	$14.25^{b,B}\pm0.70$	$20.23^{a,B}\pm0.19$
120 minutes	$14.5^{c,A}\pm0.33$	$17.50^{b,A}\pm0.31$	$23.44^{a,A}\pm0.24$
Thiobarbituric Reactive Acid Substance (TBA) (mg malonaldehyde/Kg)			
30 minutes	$0.54^{c,B}\pm0.05$	$0.71^{b,B}\pm0.02$	$0.89^{a,B}\pm0.02$
60 minutes	$0.61^{c,B}\pm0.04$	$0.81^{b,B}\pm0.02$	$1.20^{a,B}\pm0.02$
120 minutes	$0.81^{c,A}\pm0.03$	$1.10^{b,A}\pm0.12$	$3.29^{a,A}\pm0.12$

Data indicates means±SE

$^{a-c}$, $^{A-C}$ Means with different superscripts for temperature and time in each row and column respectively differ significantly at (*P<0.05*)

raising the temperature of storage where keeping samples at the highest temperature recorded the highest counts of fecal coliform. Although there was no significant (*P>0.05*) difference in fecal coliform between samples after 30 and 60 minutes of storage, samples stored for 120 minutes at the maximum temperature (37 ºC) showed the highest count. Isolated coliform organisms in (Figure 2) recorded that *Citrobacter diversus* was the most common isolate (43%) in examined samples followed by *Citrobacter freundii* (17%), *Serratia fonticola* (12%), *Enterobacter intermedius* (9%), *Enterobacter aerogenes* (7%), *Enterobacter cloacae* (3%), *Klebsiella oxytoca* (1%), and *E.coli* (8%). Similar results were found by El-Fakhrany et al. (2019) who examined burger sandwiches and isolate *Enterobacter cloacae*, *Citrobacter diversus*, *Klebsiella oxytoca* and *Kluyvera spp*. with incidence 69%, 15%, 8% and 8% respectively. The existence of *E.coli* in ready to eat sandwiches is an indicator that polluted water and dirty handling utensils were used. Additionally, contaminated water was used to wash vegetables attached to sandwiches such as cabbage, cucumber, and tomatoes, which were eaten fresh without heat treatment (Kaneko et al., 1999) is regarded as a health public source of *E.coli*. Furthermore, Nouran et al. (2019) proved that hand water and tap water in Giza governate were contaminated with *E.coli* by incidence 18.9% and 28.2%, Salmonella spp. by 19.4% and 19.4%, Klebsiella spp. 21% and 26.8% as well as Shigella spp. were present by 28.7% and 25.33% respectively. Generally, the presence of any members of the family Enterobacteriaceae in fast food meat sandwiches indicates bad handling and contamination during preparation and marketing (El-Fakhrany et al., 2019).

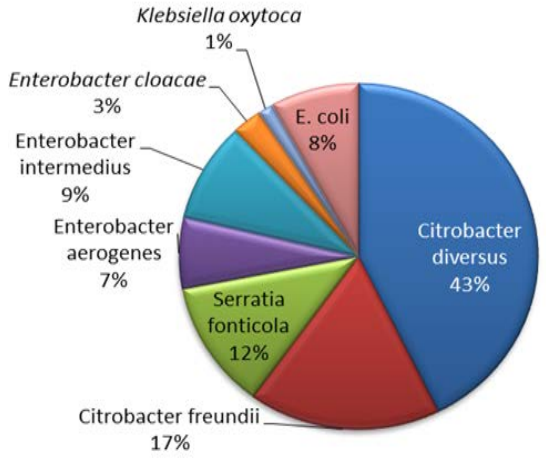

Figure 2: Incidence of coliform (%) isolates obtained from cheese burger sandwiches kept at 37 ºC for 120 minutes

The results of total staphylococci count (log_{10} CFU/g) of examined sandwiches show a significant (*P< 0.05*) increase among sandwiches at different storage temperatures and storage times. Moreover, keeping leftover sandwiches at a high temperature (37 ºC) for a long storage period (60 and 120 minutes) recorded the highest total staphylococci count which exceeds the acceptable limit (>10^4 CFU/g) mentioned by Food Standards (2018). These results were consistent with Shaltout et al. (2015) who showed that the initial staphylococci count in street vendor burger just after processing were 3.44 (log_{10} CFU/g). On the other hand, the initial total staphylococci count after 30 minutes of collecting samples were lower than those obtained by (El-Fakhrany et al., 2017) who recorded 6.97 (log_{10} CFU/g) in beef burger sandwiches. A High count of staphylococci in examined sandwiches reflects bad hygienic conditions during the preparation, processing, and marketing of sandwiches (Ahmed et al., 2019). It was stated that effective washing of hands and wearing disposable gloves during food preparation are effective methods for preventing food contamination of food *by staphylococcus aureus*. According to our study, Salmonella failed to be detected either by direct or indirect techniques. The obtained results may be due to the effect of freezing of beef burger on salmonella that causes either metabolic impairment (Ray and Speck, 1973) or structural injury in the cell wall of salmonella so it cannot grow on selective media as XLD (Barrell, 1988). While El Rahman et al. (2018) can isolate salmonella from burger sandwiches with an incidence of 8%.

Effect of changes in times and temperatures on deterioration criteria of leftover cheeseburger sandwiches

The results of the deterioration parameters of the analyzed samples of leftover cheeseburger sandwiches at varying storage temperatures and periods (Table 2) revealed that increasing storage temperature and storage duration lead to a significant (*P< 0.05*) increase in the pH of samples. Furthermore, sandwiches held at the maximum temperature (37 ºC) for the longest time (120 minutes) had the highest pH, followed by samples kept at room temperature (25 ºC) and those kept in the refrigerator (4 ºC). These findings are in agreement with the findings of Edris et al. (2012), who discovered that the pH value of beef burgers was 5.97 immediately after processing. The increase in pH caused by increasing storage temperatures (37 ºC) may be attributed to microbial development, which resulted in protein breakdown and the release of nitrogenous compounds, causing the pH to rise. In addition, high cooking temperatures, combined with a rapid heating cycle, resulted in the loss of acidic groups and the release of hydrogen sulfide, and consequently leading to an increase in pH values in sandwiches (Vasanthi et al., 2007).

Total volatile base nitrogen (TVBN) (mg/100g) showed a significant (*P< 0.05*) rise among all samples at varying storage periods and temperatures by increasing the temperature and length of holding leftover sandwiches. Furthermore, storage at the maximum temperature (37 ºC)

for the longest time (120 minutes) resulted in the highest significant (*P< 0.05*) rise in TVBN, which exceeded the allowable limit (20 mg/100g) as specified in E.S.S. (2005). The findings obtained were like those found by Edris et al. (2012), who reported that the TVBN of the analyzed beef burger after processing was 10.15 mg percent. In general, high deterioration criteria of the examined sandwiches may be attributed to the effect of freezing and thawing of beef burger which affect the shelf life of sandwiches.

Thiobarbituric Reactive Acid Substance (TBA) of examined sandwiches showed no significant (*P> 0.05*) increase in TBA values after 30 and 60 minutes. In addition, storing sandwiches at the highest temperature (37 ºC) for the longest storage period after 60 minutes at 37 ºC and after 30, 60, and 120 minutes at different storage temperatures recorded the highest TBA value (3.29 mg malondialdehyde/kg) which exceed the permissible limit defined by E.S.S. (2005) (0.9 mg malondialdehyde/kg). These findings were higher than those obtained by Edris et al. (2012) who determined that the TBA value of beef burger was 0.11 mg malonaldehyde/kg as these samples were examined immediately after purchasing and formulated with different raw materials. However, these results were lower than those discovered by El-Fakhrany (2019) who found that TBA of burger sandwiches was 7.81 mg malonaldehyde/kg. It is well known that TBA values are a very important quality index for incipient fat rancidity (Jay, 1972). When TBA values of sandwiches exceed 0.9 mg malondialdehyde/kg, the fat become rancid and cause changes in color, odor, flavor, aroma, and nutritional value of sandwiches (Kolakowska, 2003). In addition, fat oxidation resulted in the production of harmful compounds which lead to cancer and atherosclerosis in human (Pereira and Abreu, 2018). Thus, sandwiches stored under high temperature for 120 minutes must not be consumed as it endangers public health. Consequently, from the present study, to obtain safe leftover food, Once the food is cooked, do not leave it at room temperature for more than 2 hours. Moreover, if you think you cannot use leftover food shortly, put them in the freezer to slowdown the bacterial growth and fat oxidation.

CONCLUSIONS

The effect of temperature and time changes on the microbial count (APC, psychrotrophic, total coliform, fecal coliform, *E.coli*, total staphylococci, and salmonella) as well as deterioration criteria (pH, TVBN, and TBA) values of leftover cheeseburger sandwiches kept at different storage conditions. Samples categorized into three groups: the first, the second, and the third group were held at room temperature (25˚C), in a fridge at 5˚C, and the third at temperature 37˚C respectively. Results revealed that keeping leftover sandwiches at refrigerator showed the least significant bacteriological and deteriorative changes followed by samples held at room temperature 25˚C then sandwiches kept at the highest temperature (37 ˚C) for more than 30 minutes. Since, increasing the storage temperature resulted in growth of food poisoning microorganisms which consequently threaten the public health of consumers. Besides, the increase of deterioration criteria exceeding the acceptable level described by E.S.S. which indicate the occurrence of incipient deterioration as well as changes in taste, odor, and nutritional quality and safety. Thus, consumers should not eat sandwiches kept outside refrigerator for more than 30 minutes otherwise keep leftover food at freezing conditions.

ACKNOWLEDGMENTS

This research was commercially supported by the faculty of veterinary Medicine, Cairo University.

AUTHORS CONTRIBUTION

Nermeen Makram Louis Malak: Collection of Data, analysis of results, Methodology, Investigations, Resources, and Writing review.
Neveen Soliman Mohamed Soliman: Investigations, Resources, Methodology.

CONFLICT OF INTEREST

No conflict of interest

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