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QUALITY EVALUATION OF FRESH AND REFRIGERATED BEEF BURGER SOLD IN ASSIUT CITY

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ABSTRACT

The present study aimed to assess the safety and quality of the beef burger sold in Assiut city, Egypt. Hundred random samples of fresh and refrigerated beef burger (50 for each) were collected during the period from August to October 2021 from different butcher's and refrigerators of markets, respectively. Samples were subjected to sensory, physico-chemical as well as microbiological evaluation. The findings revealed that the examined refrigerated samples have low scores of sensory attributes less than the fresh ones obtained from butchers. Deterioration criteria of samples indicated low thiobarbituric acid values (TBA), their means were 0.22±0.02 and 0.25±0.03 mg malonaldehyde/kg and the mean pH values were 5.8±0.23 and 6.0±0.30, in fresh and refrigerated samples, respectively. Furthermore, microbiological evaluation: regarding fresh burger, the mean values of aerobic plate count (APC) and total yeast and mold count (CFU/g), were 8.5 × and 1.1×, respectively. Regarding the refrigerated beef burger, the mean values of APC and total yeast and mold count (CFU/g) were 4.5 × and 3.9×, respectively. The incidence of Salmonellae in all burger samples was 24%. It was 22 % and 26% in fresh and refrigerated samples, respectively, where *S. enteritidis* and *S. typhimurium* contaminated 8% and 7% of examined samples, respectively. This study could conclude a substandard production and storage system in the area, necessitating the development of new burger production methods as well as raising knowledge about sanitary beef burger production, processing, and handling.

Short title: Evaluation of beef burger sold in Assiut city

Key words: TBA; *Salmonella* spp.; beef burger; pH and APC.

INTRODUCTION

Because we can't survive without food, food quality monitoring is a critical step and directly linked to our daily life. Because the link between nutrition and health is becoming more and more of a hot topic, food manufacturers must portray their products in the best possible light to meet consumer needs for fresh, durable, and safe foods (Hassanien *et al*., 2018).

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Each year, one-third of the food produced for human use is mostly wasted owing to spoiling, which makes food undesirable for consumers (Principato *et al.*, 2021). Meat, with its moderate pH and high nutrient and moisture content, is one of the most perishable commodities among the numerous food products. The key factors that influence spoilage and nutrient breakdown in meat are microbial growth, lipid oxidation and enzymatic autolysis, that results in the development of off-odors and flavors, slime formation, and discoloration, thereby making it unacceptable for human consumption (Pellissery *et al.*, 2020)

Due to drastic lifestyle changes, consumers' need for fast food has risen dramatically in recent years (Bastos *et al.*, 2014). Burger's popularity stems from its pleasant sensory properties and practically rich source of protein with high biological value, energy, vitamins, and minerals, which has converted it into a habitually consumed fast food in several societies (Ramadhan *et al.*, 2011).

Beef burgers, on the other hand, are a 'high risk' product because pathogenic bacteria like *Salmonella* spp. or Shiga toxin-producing Escherichia coli (STEC) may contaminate the meat raw materials. Furthermore, pathogens relocated to the center of the product during mincing and mixing of the meat preparation, that is usually the point which gets the minimum heat treatment during cooking (FSAI Scientific Committee, 2018). Beef burgers have been linked to a number of outbreaks. In 2010, one of the greatest *S. typhimurium* foodborne outbreaks in France was observed, with 554 clinical cases (Guillier *et al.*, 2013). Also, there was an S. *enteritidis* outbreak in France that was associated with the consumption of beef burgers from Poland in 2015. For *Salmonella*, 2.8% of all strong outbreaks were linked to beef products (ECDC, 2014).

Refrigerated storage is the traditional method for preserving fresh meat (Kim *et al.*, 2013). Although numerous researchers have observed that fungi and psychrotrophic bacteria are frequently related to the deterioration of perishable foods at refrigerator temperatures, resulting in a shorter shelf life. Consuming contaminated refrigerated foods is also raising the global incidence of food-borne diseases (Oluwaseun *et al.*, 2018).

The most reliable indicator of meat quality, sanitary processing, and durability of meat products is the aerobic plate count (APC). High APC of mesophilic bacteria may suggest incipient deterioration rather than any significant health hazard (ICMSF, 1980).

Mold and yeast comprise a vast group of microorganisms that are widespread in nature. Contamination of meat products with different yeast and mold species is regarded as a genuine risk since it increases the likelihood of spoilage and degradation, resulting in significant economic losses and posing a public health threat due to the creation of a wide range of mycotoxins (Morshdy *et al.*, 2015; Abd El-Wahab *et al.*, 2021). Mycotoxins have been studied for their toxigenic, hepatotoxic, nephrotoxic, immunosuppressive, carcino-genic, and mutagenic properties (da Rocha, 2014).

The determination of pH is one of the critical quality aspects of meat. Changing the pH of meat has a substantial impact on its properties, including water-binding capacity, color, consistency, smell and taste, salt penetration rate and stability during storage (Okuskhanova *et al.*, 2017).

Values of thiobarbituric acid (TBA) could be a valuable quality index for determining rancidity in lipid-rich foods during storage . (Hassan & Omama, 2011). TBA is practically used for the measurement of malondialdehyde (MDA) content that is an abundant secondary product of lipid oxidation and is relatively stable compared to lipid hydroperoxides, primary products of lipid oxidation (Jung *et al.*, 2016).

Globally, Salmonella spp. are thought to be responsible for about 90 million of diarrhea-associated diseases each year, and 85 % of those cases are linked to food (Hung *et al.*, 2017). The infective dose is often between 10^6 and 10^8 cells, however even the dose of 10 cells can cause salmonellosis in some persons (Chlebicz and Śliżewska, 2018). Fatalities are mainly observed in children below the age of 4 years who are infected with serotypes enteritidis or typhimurium (de Jong *et al.*, 2012).

So, the current research was planned to evaluate the sensory, physico-chemical and microbiological profiles of fresh and refrigerated beef burger sold in Assiut city, Egypt.

MATERIALS AND METHODS

1. Collection of Samples:

A total of 100 random samples of beef burger were collected during the period from August to October 2021, where 50 of them were freshly prepared and bought from different butchers' shops and the other 50 samples were refrigerated and stored in refrigerators in different hypermarkets and meat shops in Assiut city, Egypt**.** Each sample was packed in a plastic bag and transferred immediately with a minimum period of delay to the laboratory in an icebox.

2. Sensory evaluation:

The samples were evaluated for color, odor, and taste according to Hassanien *et al.*, (2018) and Gracey J. (1986). The evaluation of beef burger samples was assessed by 5-7 members of the Food Hygiene Department (with past experience in burger processing and evaluation) to evaluate their sensory characteristics.

3. Physico-Chemical examination:

a. Determination of pH value (Garavito *et al.*, 2020).

b. Determination of Thiobarbituric acid (TBA) (Buege & Aust, 1978)

4. Microbiological examination:

a. Determination of Aerobic plate count (APC): It was performed in accordance with (ICMSF, 1996)

b. Determination of Total Yeast and mold count was carried out according to (APHA, 1966)

c. Isolation and identification of *Salmonellae*:

- Isolation as a food-borne pathogens (ISO 6579: 2002).

- Identification of suspected isolates of *Salmonella* spp. by microscopical examination, motility test and biochemical reactions according to MacFaddin, (2000)

- Serological identification of the isolated *Salmonellae* was performed in Food Analysis Center, Faculty of Veterinary Medicine, Benha University in accordance with Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (Denka Seiken Co., Japan).

5. Statistical Analysis:

All experiments were carried out in triplicate. One-way analysis of variance was performed using the SPSS program (SPSS Inc., Chicago, IL, USA) to determine the statistical significance of differences within the samples.

RESULTS

Table 1: Sensory evaluation for fresh and refrigerated beef burger samples (n=50).

Sensory Parameters	Color				Odor				Taste			
	Desirable		Undesirable		Desirable		Undesirable		Desirable		Undesirable	
Samples	No	%	No	%	No	%	No	%	No	%	No	%
Fresh	42	84	8	16	44	88	6	12	40	80	10	20
Refrigerated	32	64	18	36	30	60	20	40	27	54	23	46

Table 2: Statistical analytical results of pH values of freshly prepared, and refrigerated beef burger samples (n=50).

Burger samples	Min.	Max.	Mean ± S.E
Fresh	5.4	6.2	5.8 ± 0.23**
refrigerated	5.5	6.4	6.0 ± 0.3**

S.E= Standard error of the mean. ** Difference between Mean values is highly significant Difference ($p < 0.01$).

Table 3: Statistical analytical results of (TBA)* values in freshly prepared, and refrigerated beef burger samples (n=50).

Burger samples	Accepted samples (<0.5 MDA /kg)		Not accepted samples (>0.5mg MDA /kg)		Min.	Max.	Mean ± S.E
	No	%	No	%			
Fresh	48	96	2	4	0.1	0.53	0.22 ± 0.024
refrigerated	47	94	3	6	0.13	0.60	0.25 ± 0.025

S.E= Standard error of mean. No significant difference between Mean values ($p > 0.05$).
* Permissible limit = 0.5 MDA /kg

Table 4: Statistical analytical results of Aerobic plate count (APC) (cfu/g) in the examined samples of freshly prepared, and refrigerated beef burger (n=50).

Burger samples	Min.	Max.	Mean ± S. E
Fresh	4×10^5	10^9	$8.5\times10^7 \pm 4.4\times10^7$
refrigerated	5×10^5	3×10^8	$4.5\times10^7 \pm 1.4\times10^7$

S. E= Standard error of the mean. No significant difference between Mean values ($p > 0.05$).

Table 5: Statistical analytical results of Total yeast and mold (cfu/g) in the examined samples of freshly prepared, and refrigerated beef burger (n=50).

Burger samples	Positive samples		Min.	Max.	Mean ± S.E*
	No	%			
Fresh	41	82%	1.8×10^3	6×10^4	$1.1\times10^4 \pm 2.1\times10^3$*
Refrigerated	44	88%	10^3	2.5×10^5	$3.9\times10^4 \pm 1.2\times10^4$*

S.E*= Standard error of mean. * Significant difference between Mean values ($p < 0.05$).

Table 6: The incidence of Salmonellae in the examined samples of freshly prepared, and refrigerated beef burger

Incidence of isolated Salmonella	Samples					
	Fresh (n=50).		Refrigerated (n=50).		All samples (n=100).	
	No.	%	No.	%	No.	%
S. enteritidis	1	2%	7	14%	8	8%
S. typhimurium	4	8%	3	6%	7	7%
S. tsevie	2	4%	1	2%	3	3%
S. rissen	1	2%	1	2%	2	2%
S. infantis	2	4%	-	-	2	2%
S. chester	-	-	1	2%	1	1%
S. montevideo	1	2%	-	-	1	1%
Total	11	22%	13	26%	24	24%

Table 7: Serological identification of isolated Salmonellae

Identified strains	Group	Antigenic structure	
		O	H
S. enteritidis	**D1**	1,9,12	g,m : -
S. typhimurium	**B**	1,4,5,12	i : 1,2
S. tsevie	**B**	1,4,12	i : e,n,z15
S. rissen	**C1**	6,7,14	f,g : -
S. infantis	**C1**	6,7	r : 1,5
S. chester	**B**	1,4,5,12	e,h : e,n,x
S. montevideo	**C1**	6,7,14	g,m,s : 1,2,7

DISCUSSION

1. Sensory evaluation:

As in all foods, the organoleptic tests are generally the final guide of the quality from the consumer's perspective. As it is beneficial to make a comparison between sensory evaluation for fresh and refrigerated beef burger samples. The findings in table (1) revealed that the examined refrigerated samples have low scores of sensory attributes less than the fresh ones obtained from butchers' shops.

Regarding fresh burger: color, odor and taste, the percentage of undesirable samples were 16, 12 and 20 %, respectively.

Regarding refrigerated burger: color, odor and taste, the percentage of undesirable samples were 36, 40 and 46, respectively. These findings were higher than those obtained by (Hassanien *et al.*, 2018) from minced meat that were 20, 20 and 16 % respectively.

2. Physico-Chemical evaluation:

2.1. Determination of pH value:

The quality can be tested by detecting the pH value of the meat. The pH measurement of spoiled meat may be considered an indirect measurement of the accumulation of ammonia, which suggests muscle deterioration (Mu *et al.*, 2021).

As shown in table (2) the mean values of pH of examined freshly prepared and stored refrigerated beef burger samples were 5.8 ± 0.23 and 6.0 ± 0.3, respectively. It is worth mentioning that the difference between their mean values was highly significant, where P-value = 0.002 ($p < 0.01$). Shaltout *et al.* (2016) illustrated that changes in pH values may be due to endogenous enzymes and microbial load which may cause protein hydrolysis with the appearance of alkaline groups during the storage time. A similar mean pH value was recorded by Hassanien *et al.* (2018) (5.8). lower result was 5.60±0.05 Malak and Abdelsalam, (2021).

2.2. Determination of Thiobarbituric acid (TBA):

TBA has been used as an index to assess the amount of secondary lipid oxidation products closely attributed to meat sensory quality (Hu *et al.*, 2015), and a 0.5-mg MDA/kg meat was considered a threshold of rancidity perception by consumers and closely pertaining to the undesirable off-odor of meat (Sheard *et al.*, 2000). Table (3) revealed that 94% of all our samples had TBA readings below 0.5 mg MAD/kg, so microbial spoilage is responsible for the development of undesirable odor in other samples (Ghaderi-Ghahfarokhi *et al.*, 2016). No significant difference between Mean values ($p > 0.05$). Research recorded higher TBA values (mg MDA/kg) (0.66±0.02) by Malak and Abdelsalam, (2021) and (0.44) by Hassanien *et al.* (2018).

3. Microbiological evaluation:

3.1. Aerobic plate count (APC):

The results in table (4) recorded that, the mean values of APC (cfu/g) of freshly prepared, and marketed refrigerated beef burger samples were $8.5\times10^7\pm4.4\times10^7$, and $4.5\times10^7\pm1.4\times10^7$, respectively. Results demonstrated that fresh burger were highly contaminated with aerobic mesophilic bacteria however, lower results were found in frozen burger samples examined in many studies. These results agreed with those of Salem *et al.* (2018) who found that APC (cfu/g) was $2.15\times 10^7\pm 5.36\times 10^6$ in fresh meat, and lower APC ($1.63 \times 10^4 \pm 5.53 \times 10^3$) in the frozen burger. Ragab *et al.* (2016) recorded a higher value of APC (cfu/g) in fresh minced meat (6.6 x 10^8) and a lower value in the frozen burger (3.1x 10^5) which was obtained also Assiut governorate. There are nearly similar results also obtained by Tekinsen *et al.* (1980) (8.4x10^7 cfu/g.) from fresh minced meat in Ankara. A higher result was reported by Malak and Abdelsalam, (2021) (7.69 log10 cfu/g) from burger in Egypt, Gonulalan and Kose, (2003) (5.3x10^9 in minced meat).

3.2. Total yeast and mold:

In our study, the incidence of yeast and mold in all burger samples was 85%. The results in a table (5) showed that it was 82 % and 88%, with mean values of

$1.1\times10^{4}\pm 2.1\times10^{3}$ and $3.9\times10^{4}\pm 1.2\times10^{4}$ in fresh and refrigerated samples, respectively. There was a significant difference between them where P-Value = 0.03 ($p < 0.05$). APHA, (2001) illustrated that food spoilage at refrigerator temperatures is commonly caused by fungi. When low water activity, high acidity, or packing conditions favor their growth over bacteria in foods, they become the dominant cause of refrigerated food spoiling (Oluwaseun *et al.*, 2018). A higher incidence rate of 100% (60/60) and higher average value ($2.7\ x10^{7}$ cfu/g) were obtained by Erdem *et al.* (2014) in fresh minced meat. On the other hand, lower results were recorded by Direkel *et al.* (2010) found that the mean values of APC, yeast and molds detected in the meat samples were $4.7x10^{4}$ cfu/g, and $2.3x10^{3}$ cfu/g, respectively, and nearly agreed with results obtained from burger by El-Tawab, (2014) $3.06\ x\ 10^{4}\pm 0.92\times10^{4}$ cfu/g. and Salem *et al.* (2018) $1.63\times10^{4}\pm 5.53\times10^{3}$.

3.3. Prevalence of Salmonella

Salmonella spp. may survive in variable conditions. They pose a great threat to the food industry because they can adapt and grow at temperatures ranging between 8 and 45 ◦C (optimum temperature 37 ◦C) and at the pH of the environment from 4.0 to 9.5 (optimum pH 6.5–7.0) (Chlebicz and Śliżewska, 2018).

The findings in table (6) showed that the incidence of Salmonellae in the examined samples in the fresh burger was 22% (11/50), while a higher incidence of 26% (13/50) was in refrigerated samples with an overall incidence of 24% (24/100) among all examined samples. Serological identification of Salmonellae in tables (6) and (7) showed that 8%,7%,3%,2%,2%,1% and 1% of the examined samples were contaminated with *S. enteritidis, S. typhimurium, S. tsevie, S. rissen, S. infantis, S. chester* and *S. montevideo*, respectively.

Our finding of Salmonella recovery is approximately near to that reported by other researchers. For instance, 35% in burger where *S. typhimurium* was 17.5% out of total isolated Salmonella Elbayoumi *et al.* (2021), 24% in minced meat Eltanani and Arab (2021) and, 23.3% and 12.2% in minced meat and frozen burger, respectively, Sallam *et al.* (2014). A much higher prevalence of 62% was reported in beef samples, in Vietnam Van *et al.* (2007). Conversely, a much lower prevalence of Salmonella was reported in minced meat and frozen beef burger which were 2 (8%) and 0 (0%) Ibrahim *et al.* (2020).

Our study revealed that the examined stored refrigerated burger samples have low scores of sensory evaluation and worse microbiological and physico-chemical quality than freshly prepared samples. According to previous research, the average concentration of viable cells in refrigerators is roughly 7.1 $\log^{10}$ cfu/cm^{2}. Furthermore, spoilage-causing or pathogenic micro-organisms were found in more than half of the evaluated refrigerators, which can grow at low temperatures and reduce shelf life or potentially harm consumers' health (Clarence *et al.*, 2009).

Finally, our findings obtained from fresh and refrigerated burger were nearly close to their counterparts from fresh minced meat rather than frozen burger samples which examined in most research. That is because most pathogens don't really replicate in freezing conditions, and many of them die as a result of the failure of their enzymes to maintain normal cell activity. Pathogens also require water to grow, and freezing converts the available water into solid ice crystals (Akhtar *et al.*, 2013).

CONCLUSION

Finally, the current study allows us to conclude that the possibility of contamination of meat products with such microorganisms remains a public health and economic problems. The achieved study reflected that fresh burger was a highly contaminated product that may be considered a reliable index of fecal contamination and improper handling during processing. Consequently, strict maintenance of good practices during processing, strengthened by maintaining the cold chain during transport, distribution and storage is of central importance to ensure both public health and food quality.

Despite low temperatures, hygienic designs and cleaning recommendations, refrigerators can be hot spots for bacteria and fungi. So, care must be taken in the consumption of refrigerated foods most especially after a long period. Therefore, to improve the hygienic quality of raw meat, proper cooking of burger, avoiding post-cooking contamination, and high-quality raw materials must be taken into consideration. Also, separation of raw unprocessed meat from meat products, good hygienic practices, and application and implementation of the HACCP system especially during preparation and serving should be applied.

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تقييم مدي جودة البرجر البقري الطازج والمبرد المباع في مدينة أسيوط

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الهدف من البحث هو إجراء تقييم سلامة وجودة برجر اللحم البقري المباع في مدينة أسيوط ، مصر. تم جمع مائة عينة عشوائية من برجر اللحم البقري الطازج والمبرد (50 لكل منهما) من مختلف محلات الجزارة وثلاجات الأسواق والمتاجر ، على التوالي. خضعت العينات للتقييم الحسي والفيزيائي والكيميائي وكذلك الميكروبيولوجي. أظهرت النتائج أن العينات المبردة التي تم فحصها تحتوي على درجات منخفضة من الصفات الحسية أقل من العينات الطازجة التي تم الحصول عليها من الجزارين. أشارت معايير مدى صلاحية العينات إلى قيم منخفضة لـ (TBA) حمض الثيوباربيتيوريك ، وكانت متوسطاتها 0.22 ± 0.02 و 0.25 ± 0.03 مجم مالونالديهيد / كجم وكان متوسط قيم الأس الهيدروجيني 5.8 ± 0.23 و 6.0 ± 0.30 ، في العينات الطازجة والمبردة ، على التوالي. علاوة على ذلك ، التقييم الميكروبيولوجي: فيما يتعلق بالبرجر الطازج ، كان متوسط القيم لعد البكتيريا الهوائية (APC) وإجمالي عدد الخميرة والعفن 8.5×10^7 و 1.1×10^4 (CFU / g) ، على التوالي. فيما يتعلق ببرجر اللحم البقري المبرد ، كان متوسط القيم لعدد البكتيريا الهوائية وإجمالي عدد الخميرة والعفن4.5×10^7و 3.9×10^4 (CFU / g)، على التوالي. سجلت نسبة الإصابة بالسالمونيلا في جميع عينات البرجر 24٪. بنسبة 22٪ و 26٪ في العينات الطازجة والمبردة على التوالي ، حيث وجدت العترات S. enteritidisو S. typhimurium في 8٪ و 7٪ من العينات المفحوصة على التوالي. يمكن أن نستنتج من هذه الدراسة أن نظام إنتاج وتخزين برجر اللحم البقري كان دون المستوى في المنطقة ، مما يستلزم تطوير طرق جديدة لإنتاج البرجر بالإضافة إلى زيادة المعرفة حول إنتاج برجر اللحم البقري الصحي ومعالجته ومناولته .