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Effect of Pasteurization and Gamma irradiation on some pathogens and sensory characteristics of milk

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Abstract

The aim of this study was to compare between the efficiency of pasteurization and Gamma irradiation treatments for their antimicrobial effects on three important foodborne pathogens including L. monocytogenes, S. aureus and E. coli in milk. Additionally, the effect of these treatments on sensory characteristics of milk were also monitored before and after treatments. Milk samples were inoculated with the reference strains of L. monocytogenes "ATCC7644", E. coli "ATCC25922" and S. aureus "ATCC6538" with the inoculated dose of $10^7 Log_{10}$ cfu/ml milk (Control sample). Milk samples were subjected to pasteurization at 63°C for 30 minutes and to gamma irradiation at concentration of 2, 3, 4 and 5 kGy. A three trials based experiment was designed to each treatment and statistical analysis was performed. The obtained results showed that pasteurization process was the most significant result as all inoculated pathogens under study were completely eliminated. Otherwise, the organisms could not be detected any more after application of pasteurization, while all examined samples are positive for inoculated pathogens after gamma irradiation treatments even with the highest dose of 5 KGy, the reduction increased with the increasing of irradiation dose. Moreover the obtained results reveal that Gamma irradiation had no adverse effect on sensory characteristics of milk while pasteurization induces adverse effects.

Key words: Staphylococcus aureus, Escherichia coli, L. monocytogenes, pasteurization, gamma irradiation, milk.

Introduction

Milk is a food with high nutrient medium, high water activity and neutral pH. These characteristics makes the milk an ideal medium for microbial growth (**Dahmer, 2006**), those organisms causing its fast spoilage and reduction of its shelf-life as well as some of these microorganisms may be pathogenic that lead to public health significance for consumers.

The thermal treatment is the conventional method applied to reduce the microbial load in milk, it is very simple and effective, but results in several sensory and nutritional loss in the product, especially when thermal treatment is carried out in low quality milk, which presents high microbial load (**Pedras, et al. 2012**).

Traditional thermal treatments are a cornerstone of the food industry providing required safety profiles and extensions of shelf-life. However, such treatments may lead to losses of desired organoleptic properties and damage to temperature labile nutrients and vitamins. Consequently, the food industry has long sought alternative or synergistic approaches to provide the treatment objectives. Novel thermal and non-thermal technologies have been designed to meet the required food product safety or shelf-life demands while minimizing the effects on its nutritional and quality attributes.

An alternative method to reduce the milk microbial load is using substances that inactivate microorganisms without affect milk safe and quality (e.g. ozone, irradiation, peroxide and CO_2). The use of these substances can reduce the binomial of time and temperature applied in milk pasteurization due the lower counts of microorganisms in milk. Additionally, these substances can limit the enzyme production by microorganisms during its storage before thermal treatment, improving the stability of milk protein during its shelf-life (**Cavalcante et al. 2013**).

Some of non-thermal technologies as potential alternatives to thermal processing of foods include membrane filtration, osmotic dehydration, pulse electric field, ultrasound, ionizing radiation, high pressure, active packaging and ozone treatment (**Ohlsson & Bengtsson, 2002** and **Piyasena, et al. 2003**).

The most extensively researched and promising non-thermal processes appear to be Gamma irradiation has long been developed and researched and it has high potential in producing safe and nutritious food. Advantages over conventional methods include improved retention of quality and nutritional parameters, shorter processing times and higher yields.

Gamma irradiation technology uses high energy gamma rays that are emitted by radioactive Cobalt 60 or Cesium 137. These radioactive sources are produced in commercial nuclear reactors and have a long half-life that makes them useful for commercial installation.

Food irradiation is a preservation process exposing food to high energy rays to improve

product safety and shelf life. It could be used to replace chemical preservatives as well as thermal treatment.

The use of gamma irradiation in dairy product is considered as one of the most important peaceful application of nuclear energy (**FDA, 1997** and **WHO, 2005**). There was no hazard caused by irradiation up to 10 kilo grey which could not cause cancer, genetic mutation or tumors (**Mason, 1993; Ordonez et al. 1999; Sofos, 2002; Mehran et al. 2005** and **Steel, 2006**). Therefore, hospitals use irradiated food for patients with severely impaired immune system (**konteles et al., 2009**).

Material and methods

Experimental design

A three trial based experiment was designed to compare between thermal (Pasteurization) and non-thermal (Gamma irradiation) effect against reference strains of Staphylococcus aureus, Escherichia coli and Listeria monocytogenes.

Collection and preparation of samples

Ultra-high-temperature (UHT) skim milk samples were collected and prepared for detection the effects of pasteurization and gamma irradiation on sensory attributes of milk and on the count of the inoculated pathogens in milk.

Samples of milk were previously treated by boiling to ensure the complete elimination of any other microorganisms.

Preparation of bacterial inoculum

The pathogens standard strains used were Listeria monocytogenes (ATCC7644), Escherichia coli (ATCC25922) and Staphylococcus aureus (ATCC6538). The standards strains used in this experiment were confirmed to be active and of high performance by isolation and identification of the lyophilized beads.

The standard strains were obtained from media preparation and performance unit-Department of Food Hygiene, Animal Health Research Institute, Dokki, Giza-Egypt.

Each frozen culture was thawed and a loop full (~ 10µl) was suspended into 9 ml of Tryptic Soy Broth (MERK, 105459 | Tryptic Soy Broth) at 35°C, then incubated at 37°C for 16 hours in order to achieve stock culture that was approximately 10^7-$10^9 Log_{10}$ cfu/ml after 16 hours, a loopfull of each tested microorganisms was then transferred from the broth and streaked onto Tryptic soy agar slants (MERK, 105458 | Tryptic Soy agar). The slants were incubated for 16 hours at 37°C.

Prior to using, the prepared strains were subcultured by suspending a loopfull from the agar slants into 9 ml of Tryptic Soy broth and incubated for 16 hours at 37°C **(Edwards, 2012)**, where the count reached an approximately $10^9 Log_{10}$ cfu/ml.

Two ml of prepared strain which contained 10^9 cfu/ml was mixed with 200 ml of milk sample to obtain an approximate concentration of 10^7cfu/ml from each strain according to the following formula:

C1V1=C2V2

C1=Initial concentration

V1=Initial volume

C2=Final concentration

V2= Final volume

Laboratory pasteurization of inoculated milk samples (APHA, 1985)

Inoculated milk samples were transferred to sterile glass flasks and heated in calibrated water bath to 145°F (62.8°C), held for 30 minutes followed by immediate cooling. The pasteurization process was carried out in triplicate. Pasteurized non-inoculated milk sample was used as control sample and calibrated thermometer was put in this control sample to monitor and insure pasteurization temperature. This sample was also used to examine sensory attributes of pasteurization process.

Samples of raw non-inoculated milk were used as control sample to test sensory attributes of pasteurized milk.

Radiation of inoculated milk samples

Milk samples in screw capped tubes were irradiated with 0.0, 2.0, 3.0, 4.0 and 5.0 kGy gamma irradiation at dose rate 1.774 kGy/ hour using the "Indian Gamma Chamber 4000 A" with a ^{60}Co source. The irradiation process was conducted at the National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo, Egypt. After irradiation, all samples were transferred to a refrigerator and kept at 4 °C until examination. The experiment was repeated 3 times and the average was recorded to ensure accuracy).

Non inoculated irradiated milk sample was used as control sample to test sensory attributes of irradiated milk.

Examination of treated samples

1. Microbiological Examination

1. a. Preparation of serial dilution (APHA, 2004)

One milliliter of treated, milk sample was thoroughly mixed aseptically transferred to 9 ml peptone water (**Oxoid, CM 0509**) and well mixed to obtain 1/10 dilution. One ml from the first dilution was added to 9 ml of sterilized diluents to obtain tenth fold serial dilutions. The prepared dilutions were subjected to the following microbiological examinations:

1.b. Enumeration of pathogens

From each dilution 0.1 ml was aseptically spread over the dried surface of double sets of specific medium for each pathogen:

1.b.1. Staphylococcus aureus count

From each dilution, 0.1 ml was spread onto a dry surface of double sets of Baird parker agar plates (**LABM, LAB085**). Inoculated plates were incubated at 35°C for 48 hours (**BAM, 2001**). Typical colonies of S. aureus (black shining convex colonies, 1-1.5 mm in diameter with narrow white margin and surrounded by a clear zone extending into opaque medium) were enumerated and the average number per ml was calculated.

1.b.2. Escherichia coli count

From each dilution, 0.1 ml was spread onto a dry surface of double sets of Eosin methylene blue (EMB) agar plates (**LABM, LAB061**),. Inoculated plates were incubated at 35°C for 24 hours (**BAM, 2002**). Typical colonies of Escherichia coli (dark centered and flat, with or without metallic sheen) were enumerated and the average number per ml was calculated.

1.b.3. Listeria monocytogenes count

From each dilution, 0.1 ml was spread onto a dry surface of double sets of Oxford agar plates (**LABM, LAB122**). Inoculated plates were incubated at 35°C for 48 hours (**BAM, 2003**). Typical colonies of L. monocytogenes (black with black halo) were enumerated and the average number per ml was calculated.

2. Sensory examination (Nelson and Trout, 1981)

In each experimental trial, control negative (non-treated and non-inoculated) milk sample was included to be compared with treated non-inoculated milk samples to detect effect of each treatment on sensory attributes of milk.

In case of pasteurization, raw milk sample was used to be compared with pasteurized non inoculated milk sample to detect effect of pasteurization on sensory attributes of milk.

A five trained test panel evaluation of the samples was done for the color, odor and taste characteristics (**Nelson and Trout, 1981**) after pasteurization and gamma irradiation then the average was recorded as overall sensory score ranging from 5 = very good, 4 = good, 3 = accepted, 2= dislike to 1 = very dislike.

Statistical analysis

Microbial counts and sensory values were analyzed by analysis of variance (ANOVA) using IBM SPSS statistics 20 to find differences among treatments.

Main effects were considered significance at $p<0.05$.

Results

Table 1: Statistical analytical results of the Pasteurization effect on experimentally inoculated milk samples with pathogens (log_{10} cfu/ml)

Pasteurization process at 63°C/30minutes		
Types of pathogens	Mean (log_{10} cfu/ml) ±SD	
	control	After Pasteurization
Staphylococcus aureus	7.97^{A} ±0.005	$<10 \pm 0^{a}$ (R%=100%)
Escherichia coli	7.15^{A} ± 0.035	$<10 \pm 0^{a}$ (R%=100%)
Listeria monocytogenes	7.65^{A} ±0.039	$<10 \pm 0^{a}$ (R%=100%)

- <10 was calculated as zero count when using SPSS program
- There are significant difference ($p<0.05$) between means have the same capital and small letter in the same row

- R%=Reduction percent.

Fig. (1): The mean value of pasteurization effect on experimentally inoculated pathogens (log_{10} cfu/ml)

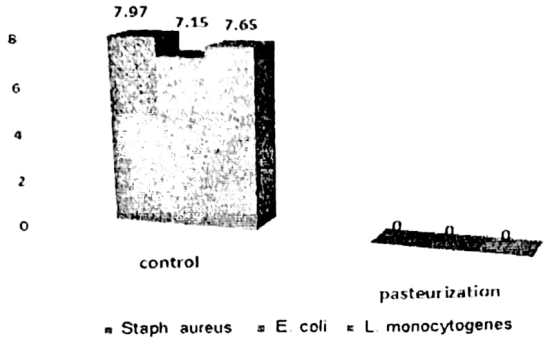


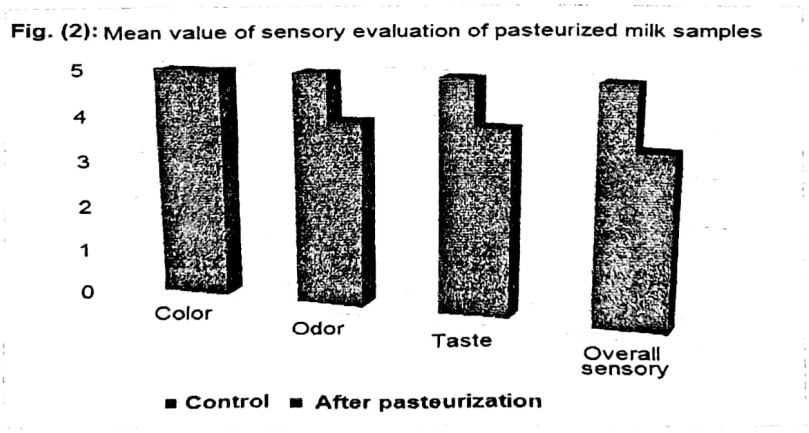
Table 2: Sensory evaluation of pasteurized milk samples

Types of samples	Sensory evaluation scores			
	Color	Odor	Taste	Overall sensory score*
Control samples	5 ±0	5 ±0^{A}	5 ±0^{A}	5 ±0^{A}
Pasteurized samples	5 ±0	4 ±0^{a}	4 ±0^{a}	3.67 ±0^{a}

* Mean of color, odor and taste.

There are significant difference ($p<0.05$) between means have the same capital and small letter

Fig. (2): Mean value of sensory evaluation of pasteurized milk samples



in the same column.

Table 3: Statistical analytical results of the effect of different gamma irradiation doses on experimentally inoculated pathogens in milk samples (log_{10} cfu/ml).

Types of pathogens	Gamma irradiation dose (Kgy)				
	Control	2 Kgy	3 Kgy	4 Kgy	5 Kgy
	Mean Logarithmic cfu/ml				
Staphylococcus aureus	7.97 ^{A}±0.005	6.67^{aB}±0.047 (R%=16%)	5.22^{abC}±0.063 (R%=35%)	4.1^{abcD} ±0.002 (R%=49%)	3.09^{abcd} ±0.103 (R%=61%)
Escherichia coli	7.15^{A} ±0.035	3.69^{aB}±0.088 (R%=48%)	3.59^{aC}±0.111 (R%=50%)	2.41^{abc} ±0.137 (R%=66%)	2.25^{abc} ±0.204 (R%=69%)
Listeria monocytogenes	7.65^{A} ±0.039	7.36^{aB} ±0.02 (R%=4%)	5.82^{abC}±0.014 (R%=24%)	3.64^{abcD} ±0.051 (R%=52%)	3.2^{abcd} ±0.174 (R%=58%)

- There are sig. difference ($P<0.05$) between the means have the same capital and small letters in the same raw.
- R%=Reduction percent.

Fig. (3):Mean value of different irradiation doses on experimentally inoculated pathogens organisms in milk samples (log_{10} cfu/ml)

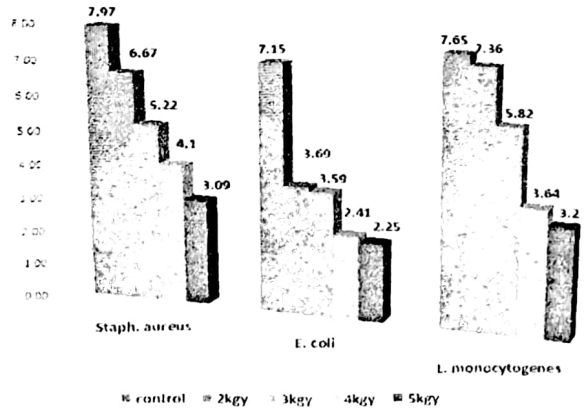


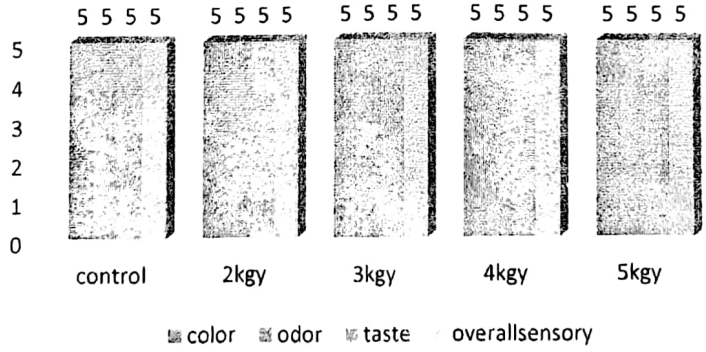
Table 4: Sensory evaluation of gamma irradiated milk samples

Gamma irradiation dose (kGy)	Sensory evaluation scores			
	Color	Odor	Taste	Overall sensory score *
Zero	5 ±0	5 ±0	5 ±0	5 ±0
2	5 ±0	5 ±0	5 ±0	5 ±0
3	5 ±0	5 ±0	5 ±0	5 ±0
4	5 ±0	5 ±0	5 ±0	5 ±0
5	5 ±0	5 ±0	5 ±0	5 ±0

* Mean of color, odor and taste.

There were no significant variations between sensory attributes in irradiated milk samples with different doses.

Fig (4): Mean value of sensory evaluation of irradiated milk samples



Discussion

Pasteurization

Data illustrated in **Table 1** and **Figure 1** showed that the pasteurization at 63°C/30 minutes induce complete elimination of inoculated strains of Staphylococcus aureus, Escherichia coli and Listeria monocytogenes. This agreed with **Oliver et al. (2005)**, **Walstra et al. (2006)**,**Peng et al. (2013)**,**Codex Alimentarius (2003)**, **Lewis** and **Heppell (2000) and Claeys et al. (2013).**

In contrary, **Lejeune** and **Rajala-Schultz (2009)** stated that while pasteurization can reduce microbial contamination, but it does not ensure that milk is sterile throughout the supply chain.

Table 2 and **Figure 2** illustrated that effect of pasteurization on sensory attributes of milk samples. Pasteurization at 63°C / 30 minutes did not affect milk color but adversely affect milk odor, taste and overall sensory. These results agreed with **Bassette, et al. (1986), Adhikari** and **Singhal (1991), Contarini, et al. (1997), Clark, et al. (2008), McSweeney** and **Fox (2009)** and **Zabbia, et al. (2012).**

Effect of gamma irradiation on Staphylococcus aureus ("ATCC6538") experimentally inoculated in milk

The results recorded in **Table 3** and **Figure 3** showed that the low level of irradiation dose at 2 and 3kGy substantially diminished the number of Staphylococcus aureus which reached to 1.3 and 2.75 log_{10} cfu/ml, respectively. While the high doses of 4 and 5 kGy induced more significant reduction which reached to 3.87 and 4.88 log_{10} cfu/ml, respectively. For the high doses of 4 and 5 kgy, the cells destruction of Staphylococcus aureus viable cell count was significantly higher ($P< 0.05$) than the control samples.

The reduction percent in the total Staphylococcus aureus count experimentally inoculated in milk samples (log_{10} cfu/ml), treated at 2, 3, 4 & 5 kGy were 16%, 35%, 49% and 61%, respectively (**Table 3**).

The obtained results declared that gamma irradiation dose at 5kGy induced the highest reduction percent (61%) in Staphylococcus aureus count which was experimentally inoculated in milk, this means that the inoculated Staph. aureus is not completely inactivated.

Nearly similar findings were reported by **El-Batawy (1999)** and **Yousef et al. (2001).**

Higher results were recorded by **Badr (2012), Kamat et al. (2000)** and **Adeil Pietranera, et al. (2003)**

Effect of gamma irradiation on Escherichia coli (ATCC25922) experimentally inoculated in milk

The results recorded in **Table (3)** and **Figure (3)** showed that gamma irradiation dose at 2 kGy resulted in significant reduction in viable cell count of Escherichia coli ($P<0.05$) which reached 3.46 log_{10} cfu/ml while gamma irradiation dose of 3kGy induced also a significant reduction which reached 3.56 log_{10} cfu/ml, therefore, there was no significant difference between 2 and 3 kGy on reduction of Escherichia coli count. While gamma irradiation at dose 4kGy induced more significant reduction which reached 4.74 log_{10} cfu/ml finally gamma irradiation dose at 5 kGy induced significant reduction which reached 4.9 log cfu/ml. There was no significant difference ($P>0.05$) between 4 and 5 kGy on Escherichia coli count reduction.

The reduction percent in the total Escherichia coli count experimentally inoculated in milk samples (log_{10} cfu/ml), treated at 2, 3, 4 & 5 kGy were 48, 50, 66 and 69%, respectively (**Table 3**). Therefore, gamma irradiation dose at 4 and 5kGy induced highest reduction in count of Escherichia coli were experimentally inoculated in milk. These results didn't agree with **Kim et al. (2005)**

Effect of gamma irradiation on L. monocytogenes (ATCC7644) experimentally inoculated in milk

The results recorded in **Table 3** and **Figure 3** illustrated the effect of gamma irradiation dose at 2 kGy which found to be a cause of significant reduction in viable Listeria monocytogenes cell count of ($P<0.05$) which reached 0.29 log_{10} cfu/ml, while gamma irradiation dose at 3kGy induced more significant reduction which reached 1.83 log_{10} cfu/ml. Moreover, gamma irradiation dose at 4kGy induced more significant reduction which reached 4.01 log_{10} cfu/ml, finally gamma irradiation dose at 5 kGy induced the highest significant reduction which reached 4.45 log_{10} cfu/ml.

The reduction percent in the total Listeria monocytogenes count experimentally inoculated in milk samples (log_{10} cfu/ml), treated at 2, 3, 4 & 5 kGy were 4, 24, 52 and 58%, respectively (**Table 3**). Therefore, gamma irradiation dose at 5kGy induced highest reduction in count of Listeria monocytogenes experimentally inoculated in milk. This didn't agreed with **Badr (2012), Foley et al. (2002).**

Sensory evaluation of gamma irradiation

(Table 4 and Figure 4) showed the effect of gamma irradiation on the sensory attributes of milk samples. The obtained results indicated that gamma irradiation doses at 2, 3, 4 and 5 kgy didn't affect milk color, odor, taste and overall sensory.

These results agreed with that reported by **Badr (2012), Aly et al. (2012), El -Batawy (1999), Yousef et al. (2001), Hamam (2005)** and **Rosenthal et al. (1983)**

On the other hand, these results didn't agree with **Thakur** and **Singh (1993), Fan** and **Thayer (2002) Bongirwar** and **Kumta (1967) (Jones** and **Jelen, 1988**).

Conclusion

The results obtained in this study support that gamma irradiation is not an effective method for the reduction of pathogens in fluid milk if compared with pasteurization. Also, gamma irradiation can be applied to improve the microbial safety of milk without adverse effects on their sensory acceptability. Low-dose of gamma irradiation and high log of pathogens used in this study may be the cause of the less destructive effects of gamma irradiation. Further study is needed to determine the effect of higher doses which may be applied in milk industry.

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الملخص العربى

تاثير البسترة و اشعة جاما على بعض الميكروبات و الخصائص الحسية للحليب

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الهدف من هذه الدراسة هو المقارنة بين تاثير المعاملات الحرارية (البسترة) و الغير حرارية (أشعة جاما) من حيث تاثيرهم على سلامة وجودة الحليب السائل وذلك بدراسة تأثير كل منهما على ثلاثة من اهم الميكروبات التى تنتقل عن طريق الغذاء وهم الميكروب العنقودى الذهبى ، الايشيريشيا كولاى والليستيريا مونوسيتوجين فى الحليب السائل ، إضافة الى دراسة تاثير هذه المعاملات على الخصائص الحسية لعينات اللبن. تم تجميع عينات اللبن و حقنها بعترات مرجعية من الميكروب العنقودى الذهبى "ATCC6538" ، الايشيريشيا كولاى "ATCC25922" والليستيريا مونوسيتوجين "ATCC7644"، حيث وصل العد البكتيرى لهذه العترات 10^7 خلية/ مل لبن تقريبا (العينة الضابطة). كانت هذه المعاملات كالاتى: البسترة عند 63 درجة سيلزيوس لمدة 30 دقيقة و الاشعاع باشعة الجاما عند 2، 3، 4 و 5 كيلو جراى. و قد تم تصميم هذه التجربة على اساس اجراء ثلاث محاولات لكل معاملة و اجراء التحليل الاحصائى لها. و لقد اظهرت النتائج ان معاملة العينات بالبسترة كانت الاكثر تاثيرا من بين جميع المعاملات حيث انها قضت على جميع الميكروبات المحقونة ، بينما كان تأثير أشعة جاما محدود بحيث تواجدت الميكروبات الممرضه محل الدراسه فى جميع العينات. كما أوضحت الدراسة التأسير السلبي للبستره على الخواص الطبيعيه للحليب وقد وضح ايضا من خلال الدراسة ان الاشعاع باشعة الجاما ليس له تاثير سلبى على الخصائص الحسية للبن فى حين ان البسترة كان لها تاثير سلبى على الخصائص الحسية للبن.