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HACCP system application to enhance milk quality in some collecting centers at El Menofiya governorate, Egypt

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ABSTRACT

Milk contains biological, physical and chemical hazards that have bad effects on human health & milk shelf life. So, aim of this study was to enhance milk quality by building up a HACCP system in milk processing plant. HACCP system application was evaluated by scanning the bacteriological quality, physicochemical examination (fat, pH, total volatile basic nitrogen, thiobarbituric acid & titratable acidity), Aflatoxin M1 and sensory attribute of collected milk samples from different suppliers. The results showed a significant difference in the bacteriological, physicochemical and sensory attributes after HACCP application. HACCP application controlled different hazards during milk production that reflects on milk quality.

INTRODUCTION

Milk is the most enriched food with vitamins, minerals, protein, fat, carbohydrates and vital elements that enhance growth and development **(Sharabi et al. 2018)**. Milk must be sterile inhealthy udder, a small amount of microorganisms (mainly lactic acid bacteria) usually gather in the udder. However, potential exposure to exogenous contaminants promotes complex micro biota derived from various sources **et al. 2021**). Contamination of raw milk depends on the number and types of organisms that canposeahealth risk, as determined by the presence of micro-organisms and bacterial pathogens **(Alqeer, A. and Zelalem, T.2022).** An important source of contamination of raw milk is strictly animal related as presence of fecal or dirty residues on udder skin surface and subclinical or clinical mastitis **(Ürkek et al. 2017).**Another type of hazards that affect milk quality is its contamination with aflatoxin M1. Aflatoxin M1(AFM1)is a hydroxylated metabolite of aflatoxin B1 (AFB1) that may occur in milk (**Alshannaq & Yu 2017**).

The current food hygiene rules is protection of products against food contamination, this according to a 2009 report from the EU Member States about the application of the hygienic Regulations **(EU, 2009).** Among the long food supply chain from producer to consumer, many food safety and quality issues takes place such as HACCP system that can measure and eliminate different hazards in products to obtain safe and high-quality dairy products **(Popa & Constantinescu 2021).**

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So, the purpose of this study was to evaluate the effect of HACCP application on milk quality at different milk collecting centers, assess the enhancement of milk bacteriological, physicochemical and sensory attributes after application of HACCP, and evaluate reflection of HACCP application on aflatoxin residues in milk.

Materials and methods

HACCP system was designed and implemented in milk collecting center, El-Menofiya governorate following the twelve steps in the application of HACCP, including the seven principles, designed by **Cullor (1995).**

Samples were collected from Two main points; (i) at reception of raw milk from suppliers, (ii) from milk bulk tank, (50) Swaps from workers and surfaces samples were examined twice (before and after HACCP implementation)

(i) At reception of raw milk from suppliers.

California mastitis test (CMT)

The CMT was performed on each quarter as described by **Schalm and Noorlander(1957).** The results were recorded as suggested by **Leach et al. (2008)** and were arranged as the following: negative (score 0), trace reaction (no gel formation, score 1(+), weakly positive score 2(++), strongly positive (clear gel formed, score 3 (+++).

Fresh raw milk from different suppliers (total of 100 samples) was collected to be examined for mastitis by California mastitis test (CMT) as described by **Schalm and Noorlander (1957).**

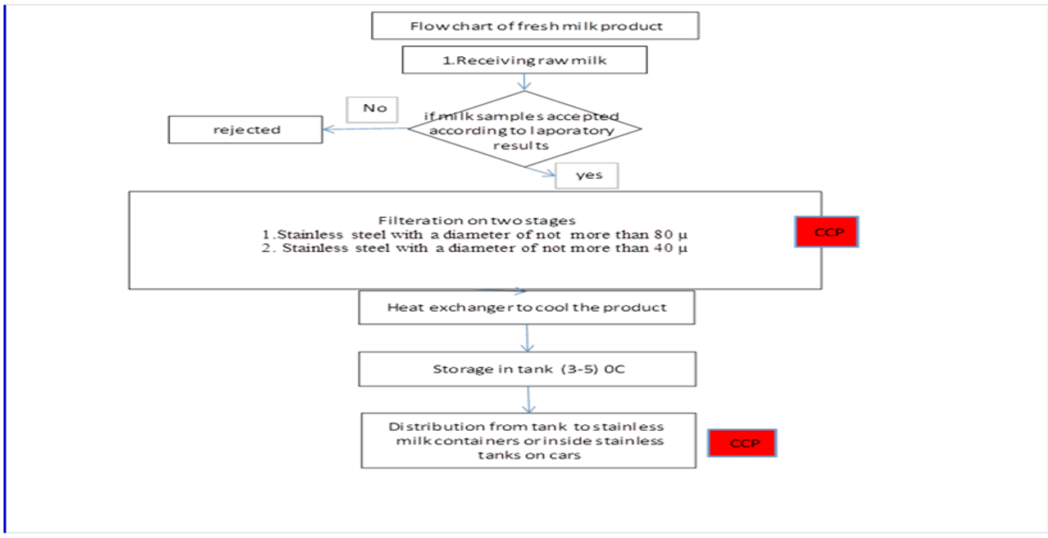

Fig.1 flowchart for CCP at milk collection center

(ii) From milk bulk tank

RESULTS

Table 1. California mastitis test (CMT) evaluation of received meat (n= 400)

Samples examined	Positive samples	Negative samples	Positive (%) +	++	+++
Before HACCP	300 (75%)	100 (25%)	90(30%)	125(41.7%)	85 (28.3%)
After HACCP	160(40%)	240 (60%)	42(26.3%)	70(43.7%)	48(30%)

Table 2. Bacteriological examination for milk samples at receiving point and bulk milk tank

Quality tests	Before HACCP	After HACCP
APC (log cfu/ml)	7.39^{**}±0.05	4.55^{**}±0.03
Coliforms (log cfu/ml)	1.75^{**}±0.04	1.21^{**}±0.02
Staphylococcal count (log cfu/ml)	1.9^{**}±0.05	1.37^{**}±0.06
Individual bacterial count (log cfu/ml)	8.2^{**}±0.9	5.11^{**}±0.11
Somatic cell count (cell/ml)	$2\times10^{6}\pm7\times103^{**}$	$2.5\times10^{5}\pm1.5\times10^{2**}$

All samples were negative for *Salmonellae*.
** = Results are significantly different at (P < 0.01).

Table 3. Bacteriological examination for swaps that collected from workers and surfaces before and after HACCP system application.

Quality tests	Samples (swaps)	Before HACCP	After HACCP
APC (log cfu/100cm)	Workers	1.75^{**}±0.08	1.4^{**}±0.02
	Surfaces	1.85^{**}±0.06	1.13^{**}±0.04
Coliforms (log cfu/100cm)	Workers	1.41^{**}±0.11	ND
	surfaces	1.54^{**}±0.2	ND
_Staphylococcal count (log cfu/100cm)	Workers	1.65^{**}±0.08	ND
	surfaces	1.82^{**}±0.22	1.5^{**}±0.19

All samples were negative for Salmonellae.
Values expressed as Mean ± SE
** = Results are significantly different at (P<0.01).
ND= not detected

Table 4. physicochemical screening of tank bulk milk using Milkoscan (n= 50)

Groups	Before HACCP	After HACCP
Fat (%)	2.7^{*} ± 0.99	3.4^{*}±0.2
pH	6.8^{*}±0.016	6.6^{*}±0.04
Total volatile basic nitrogen (TVB-N)	20.6^{*}± 1.02	16.1^{*}±0.75
Thiobarbituric Acid	0.98^{*}±0.26	0.49^{*}±0.12
Titratable acidity (%)	0.37^{*}±0.03	0.22^{*}±0.02

Table 5. Aflatoxin M1 (ppt) screening for milk from suppliers and tank bulk milk

Groups	Before HACCP	After HACCP
Aflatoxin M1 (ppt) (suppliers)	46^{**}± 4	21^{**}± 6
Aflatoxin M1 (ppt) (bulk milk tank)	50^{**}±5	32^{**}±3

Values expressed as Mean ± SE
** = Results are significantly different at (P < 0.01).

Table 6. Sensory evaluation for tank bulk milk before and after HACCP implantation (n=50)

Groups	Before HACCP	After HACCP
Flavor (40)	25 ± 5	39 ± 0.25
Color (40)	29 ± 3	38 ± 0.75
Appearance (10)	5 ± 1	9 ± 0.5

(ii) From milk bulk tank

One hundred raw milk samples(approximately 250 ml) from bulk tank over different days collected to be examined and evaluated before and after HACCP application.

Bacteriological evaluation

Aerobic plate count (log) was applied according to **ISO 4833-1 /2013,** Somatic cell count (cell/ml) was applied according to **ISO 13366-2:2006,**Coliforms count (log cfu/ml) following **ISO 4832:2006,** and Staphylococcal count (log cfu/ml) according to **ISO 6888-3:2003**. Colonies were counted and the counts were expressed as $\log^{10}$cfu/ml.

Physicochemical evaluation

Fat was determined using MilkoScan™FT1, pH was determined using a pH meter **(Mettler Toledo, Greifensee, Switzerland)** at 20°C, total volatile basic nitrogen, thiobarbituric acid and Titratable acidity according to **AOAC (2000)** titratable acidity was determined as lactic acid by the titration method with 1/9N NaOH.

Screening of aflatoxin M1

Aflatoxin M1 was applied using VICAM™ permits the measurement of Aflatoxin M1 without the use of toxic solvents.

Sensory evaluation

Samples were carried out according to **Clark et al. (2009)** a panel test of 5 panelists (each sample) of the staff members of Food Hygiene and Control Department, Animal Health Research Institute, Shibin el kom branch. The milk samples were evaluated for flavor (40 points), color (40 points), and appearance (10 points).

Statistical analysis

The data of the present study were statistically analyzed using Graphpad Prism 8. One-way analysis of variance was performed for different parameters and means were compared by Duncan's multiple range tests with 99% and 95% confidence level **(Feldman et al. 2003).** Data expressed as mean ± SE**.**

RESULTS

California Mastitis Test (CMT)

As seen in table (1) the positive samples of CMT in dairy farm before HACCP system application were300 (75%), the positive samples decreased after application of HACCP system application become 160 (40%).

Bacteriological analysis

Table (2) showed the values of bacterial examination for sample collected from supplier of milk before and after application of HACCP system. Aerobic Plate Count (APC), coliforms and Staphylococcal count showed a highly significant difference ($p<0.01$) among the sample collected before and after HACCP system applications it changed from 6.36, 1.2 and 1.9 to 4.45, 1.17 and 1.35 log cfu/ml., respectively. The bacteriological evaluation for APC, coli forms and Staphylococcal count of bulk tank milk changed from 7.39, 1.75 and 1.9 to 4.55, 1.21 and 1.37 log cfu/ml., respectively. The individual bacterial count changed from 8.2±0.9 to 5.11±0.11 (log cfu/ml) while somatic cell count changed from $2\times10^6\pm7\times103^{**}$to $2.5\times10^5\pm1.5\times10^{2**}$ (cell/ml).

Also table (3) showed that Aerobic Plate Count (APC) of swaps collected from workers and surfaces were 1.75 and 1.85 (log cfu/100 cm) before HACCP application and 1.4, 1.13 (log cfu/100 cm) after HACCP application, respectively. Coliforms count of swaps that collected from workers and surfaces were 1.41 and 1.54 (log cfu/100 cm) before HACCP application while itnot detected after HACCP application. Staphylococcal count of swaps that collected from workers was 1.65±0.08(log cfu/100 cm) before HACCP application then not detected after HACCP application. Also, Staphylococcal count of swaps that collected from surfaces was 1.82±0.22log cfu/100 cm) before HACCP application then 1.5^{**}±0.19(log cfu/100 cm) after HACCP application. *Salmonella species* failed to detected.

Table (4) demonstrates that before the implementation of HACCP, the fat percent was 2.7 ± 0.99 and increased to 3.4 ± 0.2 (p < 0. 05). Also, pH value was 6.8 ± 0.016 before HACCP application but improved to 6.6 ± 0.04 (p < 0. 05). Additionally, titratable acidity dramatically changed (p < 0.05) after the HACCP system was implemented, going from0.37± 0.03 to 0.22± 0.02.

Also, Total volatile basic nitrogen (TVB-N) changed from 20.6± 1.02 to 16.1±0.75 (p < 0.05) after the HACCP system. Furthermore, Thiobarbituric Acid changed from 0.98^{*}±0.26 to 0.49^{*}±0.12 after HACCP application.

Table (5) The values of **Aflatoxin M1** from suppliers and bulk milk tank changed from 46 and 50 (ppt) to 21 and 32 (ppt) before and after HACCP application, respectively.

Table (6) shows the sensory rating of milk samples. According to the findings, the milk sample after the HACCP system was implemented had a marginally better flavor, color, and appearance than the samples before the system was implemented. Taste, color, and appearance sensory scores went from (25, 29, and 5, respectively) to (39, 38, and9, respectively).

DISCUSSION

It is important that measures retaken to ensure that high-quality raw milk is produced from healthy animals under optimal hygienic conditions, and that appropriate control measures are applied to protect human health **(Swai and Schooman 2013). Ferronatto et al. (2018)** also examined total of 538 milk samples from 34 cows and revealed that the negative score (score 0) of California Mastitis Test (CMT) was considered to be the best cut-off point at all times. Higher CMT scores occur from changes in the proportion of leukocytes in mammary gland secretions, which may be brought on by an increase in neutrophils in infected mammary glands **(Della et al. 2011).**

High bacterial load in the raw milk might probably due to contamination, high ambient temperature, unable to maintain cold chain during transportation, and poor and unhygienic milking practices (**Kakati et al. 2021**). The Aerobic Plate Count (APC) of milk samples was higher than the maximum per-missible limit as suggested by the European Union, which has been reported to be 4 logcfu/mL. Moreover, the vendors who sell milk do not maintain the cold chains, there is a great possibility for the microorganisms to grow and multiply in the milk as it provides a con-ducive environment, such as suitable pH, high water activity, and excellent growth medium.

Before HACCP application, staff employees must be well trained about importance of H ACCP application, Good Manufacture Practices (GMP) and Good Hygienic Practices (GHP). Milk is filtered to ensure its protection from pollution (flies - sunlight - dust and dirt - rain). Also, it is better to use filters that are easy to clean and disinfect, such as those made of stainless steel.

Coliforms are routinely used as indicator to the quality of the milk and milk products as some members of *Coliforms* are responsible for production of objectionable taints in milk (**Gebril et al. 2021).** Moreover, *E. coli* in milk and milk products are evaluated as an important risk factor in terms of unfavorable hygienic conditions and fecal contamination **(Ekici and Dümen 2019).** According to **Goodarzi et al. (2020**), pH was used as a quality criterion to track the state of milk shelf life, and the best value, 6.6, indicated excellent quality milk. Lactic acid builds up due to microbial development, which also results in higher pH fluctuations (**Pereira et al. 2015**) as pHis very important in the processing of dairy products.

Greater focus should be put on improving food safety quality along the chain (e.g., through ensuring the application of Good Handling Practices like the use of food grade containers and the introduction control points) that leads to minimizing microbial load and somatic cell count **(Hasibi Zavala Nacul and Cesar Revoredo -Giha 2022).**

Different serotypes of Staphylococci were isolated from cow's milk that reared in intensive reared system. Furthermore **Lianou et al. (2021)** isolated antibiotic resistant Staphylococcal strains from bulk- milk tank and related their presence to bad hygiene also, considered that Staphylococci is a risk factor. Potentially, the genetic material of these resistant Staphylococci, which is not destroyed during the thermal processing of milk, might possibly be transferred to humans **(Schwarz et al. 2017**).

Some differences in milk quality were found to be associated with the recovery of resistant Staphylococcal isolates from the milk. These can occur because of *S.aureus*is an important pathogen for both human and animal health. In dairy herds, *S. aureus* is one of the most prevalent intramammary pathogens described around the world, including Chile (**Munoz et al. 2018**). Frequent use of disinfectants can also be applied in intensively managed flocks, and this can be associated with the development of antibiotic resistance because animals are housed and there is a need for the repeated cleaning of animal houses and equipment **(Lianou et al. 2021).**

Although milk from cows suffering from subclinical IMI caused by *S. aureus* may not show macroscopic changes, intramammary infecting bacteria can be shed in the milk and be a source of bulk milk tank (BMT) contamination. Extra mammary sources of *S. aureus*, such as biofilms on milking equipment surfaces, can also contribute to BMT contamination (**Latorre et al. 2020**). So bulk tank milk must be properly clean ant this considered risk factor in milk collection center.

The European dairy sector has changed dramatically in herd size and production level over the past decades. Dairy producers are challenged by increasing demands for improving production efficiency. Furthermore, there is increasing public concern over animal health and welfare, along with environmental issues (**Rousing et al. 2020**).

According to several research, the percentage of fat increases as the amount of bacteria in milk samples decreases (**Barrón et al. 2013**).

Cows' milk with high bacterial loads have been found to have an increase in free fatty acids; this may be due to leucocyte lipases altering the milk fat globule membrane or plasmin hydrolyzing lipoproteins, both of which may promote lipolysis (**Le Maréchal et al. 2011**).

Intramammary infection can result in reduced fat content in their milk **(Fragkou et al. 2008**). Given the strict relationship between pH and titratable acidity, this last characteristic is also an important requirement in the valuation of the dairy-technological quality of milk. Acidic groups of casein (approximately 2/5 of total acidity), acid phosphates, carbonic acid (CO_2 dissolved in milk), and other mineral anions (about 2/5 of total acidity), as well as organic acids, are the main causes of fresh milk's titratable acidity. According to **Li et al. (2022)**, titratable acidity rises when the number of microorganisms is low.

The toxicity of aflatoxin M1 (AFM1) is far less well understood. However, several countries have imposed regulatory caps on the amount of AFM1 that can be present in milk and other dairy products (**Turna and WU et al. 2021**).

Aflatoxin M1 (AFM1) action level in milk and other dairy products has been set by the US Food and Drug Administration (FDA) at 0.5 µg/L (**FDA, 2005**). While the European Union (EU) has a far tighter AFM1 standard it only permits 0.05 µg/L of AFM1 in milk **(EC, 2006)** this has resulted in cases over the past ten years of milk being dumped or production was being stopped in several European countries due to AFM1 levels that were higher than

0.05 µg/L. As shown in table (6), the value of aflatoxin M1that measured in milk samples collected from suppliers before the application of the HACCP system was 46 ± 4 ppt, but it significantly differed (P < 0.01) with the implementation of the HACCP system (21 ± 6 ppt). Also aflatoxin M1that measured in milk samples collected from bulk milk tank before the application of the HACCP system was 50 ± 5 ppt, but it significantly differed (p < 0. 01) with the implementation of the HACCP system (32± 3 ppt).AFM1 contamination of milk and dairy products might have negative financial effects for dairy producers. Rejecting products that don't adhere to aflatoxin standards at domestic or foreign markets has a direct economic impact (**Balina et al. 2018)**. About 10% of the milk samples gathered in Kenya had aflatoxin concentrations above 0.5 g/L, which, if legislation were to be passed, would cost dairy farmers $113.4 million yearly (**Kemboi et al. 2020**).

The higher values sensory score reveals a positive influence of HACCP system application.

Conclusions

With growing concerns about dairy food safety, the implementation of HACCP system become very essential. HACCP system application improves the microbiological, physicochemical and sensory quality of row milk.

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