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Effect of Different Overrun Levels on Microbial Safety of Ice Cream

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Abstract: *Three types of ice cream, different by their overrun levels, were produced and the effect of the overrun levels on the microbial safety was analyzed. Five replicates of vanilla ice creams were processed and named as Sample A, Sample B, and Sample C with overruns of 45%, 60%, and 90%, respectively. Aerobic plate counts (APC), total coliform counts (TCC), Staphylococcus aureus (SA) and yeasts & molds counts (YM) were assessed in ice cream samples along with the ice cream mix, both after Day One and Day Ten of processing and frozen storage, and the results were recorded. The microbial analysis results by using one way ANOVA and T-Test revealed that both the ice cream mix and ice cream samples were not significantly different. However, all the ice cream samples presented lower microbial counts than the mix, both after Day One and Day Ten of frozen storage; and higher overrun levels and prolonged frozen storage negatively influenced the viability of microorganisms under study. Medium overrun levels coupled with good manufacturing practices, especially after pasteurization, should be adopted in ice cream making.*
Key words*: ice cream; overrun; microbial counts; microbial safety; frozen storage*

I. Introduction

Ice Cream is popular frozen food made of milk, cream, sugar, stabilizers and flavorings [1]. Ice cream is believed to have originated in China around 2000 BC. It appeared in the United States in the early 18th century. Ice cream industry in Uganda has been developing in recent years. Different countries have different definitions of ice cream [2]. During ice cream making, air is whipped into the ice cream [3]. That amount of air incorporated is termed as 'overrun', which means the percent of expansion of ice cream achieved from the amount of air incorporated into the product. The overrun, due to air, provides proper body, texture, palatability essential to a good quality product, and affects storage stability [4]. However, the incorporation of air at different levels can affect both positively and negatively the growth and survival of microorganisms, which in turn affect the quality of ice cream and can harm the life of consumers. Despite the tremendous technological advancements, the incidents of foodborne disease associated with dairy products continue to occur [5]. Ice cream, being a good growth medium for microorganisms due to its nutrients, numerous findings of food borne incidents associated with it have been reported [6].

In a survey done in Kampala-Uganda, in 2000, on microbial contamination of the diary type ice cream, 76% of the lot of samples tested had total plate counts greater than 5×10^4cfu/g, which was above acceptable set standards by FAO and by the Uganda National Bureau of Standards. *Staphylococcus aureus* counts were also found to be above the recommended standards [7]. For all the above mentioned reasons, three types of ice cream, different by their overrun levels, were processed in Kampala, Uganda, and the effect of the overrun levels on the microbial safety was analyzed.

II. Materials and Methods

2.1. Study area

The study was conducted at Uganda Industrial Research Institute (UIRI), and in Makerere University, at the College of Veterinary Medicine, Animal Resources and Bio-security, Kampala, Uganda.

2.2. Materials

The study aimed to evaluate the effect of different overrun levels on microbial safety of ice cream. The ice cream to be tested was locally made, at the Uganda Industrial Research Institute (UIRI); it consisted of the milk ingredients (fresh milk, butter and skimmed milk powder) and non milk ingredients (sugar, vanilla, Glucose syrup and stabilizers). The liquid milk to be used was tested for freshness by applying two quick tests: Alcohol test and Clot-on- Boiling tests. Both Alcohol and Clot-on-Boiling tests were negative. After the ingredients quality examination, ice cream processing at three different overrun levels and ice cream microbial safety analysis followed. Five ice cream replicates were prepared and microbiology of each was tested at different overrun levels, both after Day One and Day Ten of frozen storage.

2.3. Processing of the ice cream

The following basic steps in the manufacturing of ice cream were followed: Blending of the mix ingredients (milk ingredients and non milk ingredients), homogenization, pasteurization, aging the mix, freezing, packaging, and hardening. The overrun level was regulated by using the air incorporation screw from the heat exchanger, at 45%, 60%, and 90% (Fig. 1).

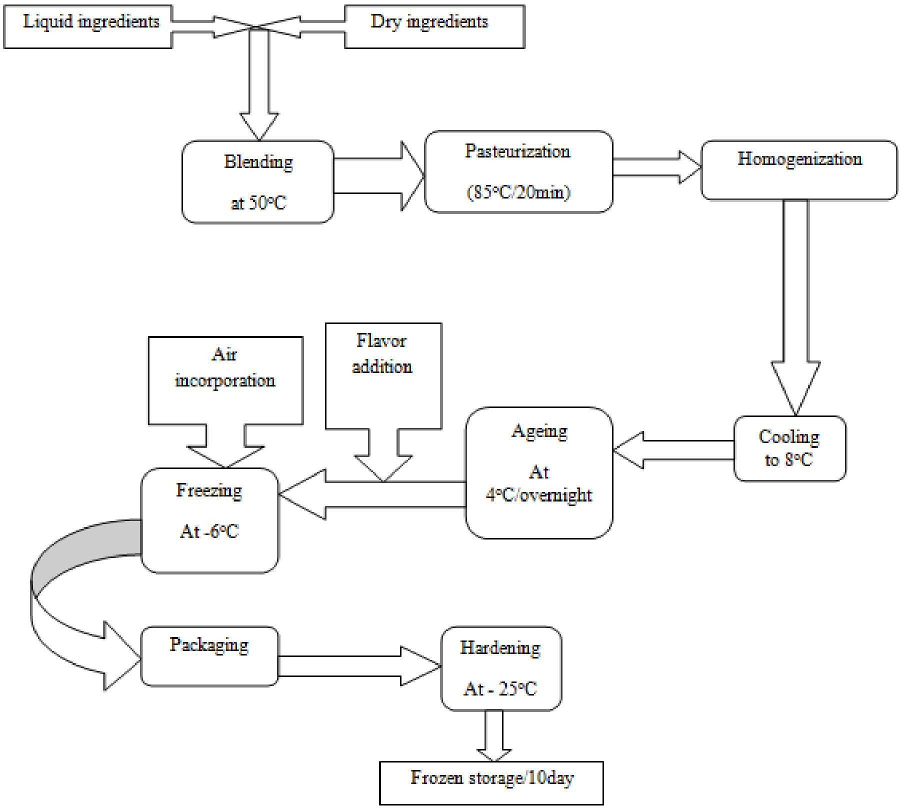

Fig.1: Ice cream processing flow diagram

2.4. Microbiological analysis

The microbial analysis of the ice cream was determined in the mix prior to the freezing/whipping and in the ice cream samples after freezing/whipping and frozen storage for 1 and 10 days. Five samples were taken from the frozen storage at UIRI, in their cups, placed in cool boxes, then transported to the microbiology laboratory at Makerere University, and tested for Aerobic Plate Counts (APC), Total Coliforms (TC), *Staphyloccocus aureus* (SA), and yeasts and molds (YM).

2.5. Statistical analysis

One way ANOVA (Analysis of Variance) and T-test were used to compare the microbial results of the ice cream mix and the ice cream samples, both after Day One and Day Ten of frozen storage.

III. Results

3.1. Microbial results of the ice cream mix and ice cream samples after Day One

The general tendency of the results after Day One of processing was that the higher the overrun level of samples, the lower the microbial counts. According to the East African Standards for ice cream ice cream is considered having unacceptable safety when the Aerobic Plate Counts exceed 4×10^4 cfu/ml; when Total Coliform Counts exceed 10^1cfu/ml; when Yeasts &Molds counts exceed 10^1cfu/ml; and when there is any presence of *Staphylococcus aureus*.

In this study, the ice cream Mix, sample A and sample C exceeded the standard limits in terms of aerobic plate counts, total coli form counts, yeasts and molds, and in terms of *Staphylococcus aureus*. Sample B exceeded the standard limits in terms of aerobic plate counts, total coli form counts, and in yeasts and molds.

However, sample B complied with standard limits in terms of *staphylococcus aureus* (TABLE 1). Referring to the same table, with the T-test analysis, the ice cream mix and all the ice cream samples were not significantly (p>0.05) different under all parameters of study, at 0.05 level of significance.

Table 1. Microbial counts and T-test results of samples after Day One

Sample	Parameter	Overall mean (cfu/ml)	p-Mix	p-A	p-B	p-C
Mix	APC	5.9×10^5	-	0.1751	0.3181	0.1945
	TCC	2.8×10^3	-	0.3782	0.3747	0.3743
	SA	2.4×10^1	-	0.5291	0.3739	0.6249
	YM	2.9×10^3	-	0.3775	0.3849	0.3762
A	APC	2.1×10^5	0.1751	-	0.1998	0.2737
	TCC	1×10^3	0.3782	-	0.3686	0.3678
	SA	6×10^3	0.5291	-	0.3739	0.3739
	YM	5.3×10^2	0.3775	-	0.4360	0.3694
B	APC	1.6×10^5	0.3181	0.1998	-	0.3289
	TCC	5.6×10^1	0.3747	0.3686	-	0.3495
	SA	0.00	0.3739	0.3686	-	0.3739
	YM	1.9×10^2	0.3849	0.4360	-	0.1317
C	APC	8.7×10^4	0.1945	0.2737	0.3289	-
	TCC	1.4×10^1	0.3743	0.3678	0.3495	-
	SA	6.2×10^1	0.6249	0.3739	0.3739	-
	YM	9×10^1	0.3762	0.3694	0.1317	-

APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeast and Molds; p-Mix: probability value for the ice cream Mix; p-A: probability value for sample A; p-B: probability value for sample B; p-C: probability value for sample C.

The one way ANOVA of microbial results analysis showed no significant (p>0.05) difference between the ice cream Mix, Sample A, Sample B and Sample C on the microbiology of all parameters under study after Day One (TABLE 2).

Table 2. One way ANOVA p-values results between all samples after Day One

Samples	Parameter	f-V	p-V
All samples	APC	0.45	0.7184
All samples	TCC	0.76	0.5322
All samples	SA	0.70	0.5656
All samples	YM	0.81	0.5049

APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeasts and Molds; f-V: calculated value; p-V: probability value

3.2. Microbial results of the ice cream mix and ice cream samples after Day Ten

The ice cream mix did not exceed the standard limits in terms of aerobic plate counts. However, it exceeded the standard limits in terms of total coliform counts, yeasts and molds, and in terms of *Staphylococcus aureus*. Referring to the same table; sample A and sample B exceeded the standard limits in terms of aerobic plate counts, total coli form counts, and in terms of *Staphylococcus aureus*. However, both of the samples did not exceed the limits in terms of yeasts and molds. Sample C did not exceed the standard limits in terms of aerobic plate counts and in yeasts and molds on one hand but it exceeded the standard limits in terms of total coliform counts and slightly in *Staphylococcus aureus* counts on the other side. From the T-test analysis, the ice cream mix and all the ice cream samples were not significantly (p>0.05) different under all parameters of this study, at 0.05 level of significance (TABLE 3).

Table 3. Microbial counts and T-test results of samples after Day One

Sample	Parameter	Overall mean (cfu/ml)	p-Mix	p-A	p-B	p-C
Mix	APC	4×10^4	-	0.4327	0.8728	0.9340
	TCC	5×10^1	-	1.00	0.7837	0.6120
	SA	1.4×10^1	-	0.6072	0.6745	0.2302
	YM	3.2×10^1	-	0.4142	0.4213	0.8358
A	APC	1.8×10^5	0.4327	-	0.4039	0.3487
	TCC	5×10^1	1.00	-	0.6686	0.4381
	SA	8×10^0	0.6072	-	1.00	0.3739
	YM	6×10^0	0.4142	-	0.4766	0.2577
B	APC	4.5×10^4	0.8728	0.4039	-	0.6508
	TCC	4×10^1	0.7837	0.6686	-	0.5291
	SA	8×10^0	0.6745	1.00	-	0.5870
	YM	1×10^1	0.4213	0.4766	-	0.2420
C	APC	3.7×10^4	0.9340	0.3487	0.6508	-
	TCC	2.8×10^1	0.6120	0.4381	0.5291	-
	SA	4×10^0	0.2302	0.3739	0.5870	-
	YM	6×10^0	0.8358	0.2577	0.2420	-

APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeast and Molds; p-Mix: probability value for the ice cream Mix; p-A: probability value for sample A; p-B: probability value for sample B; p-C: probability value for sample C.

The ANOVA of microbial results analysis showed no significant ($p>0.05$) difference between the ice cream Mix, Sample A, Sample B and Sample C on the microbiology of all parameters under study after Day One (TABLE 2).

Table 4. One way ANOVA p-values results between all samples after Day Ten

Samples	Parameter	f-V	p-V
All samples	APC	0.69	0.5736
All samples	TCC	0.15	0.9264
All samples	SA	0.49	0.6969
All samples	YM	0.46	0.7146

APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeasts and Molds; f-V: calculated value; p-V: probability value.

3.3. General comparison of microbial results, both after Day One and Day Ten

Comparing the microbial results both after Day One and Day Ten by using ANOVA, the ice cream mix, Sample A and sample C microbial count results were not significantly different under the four parameters in this study ($p>0.05$). Sample B microbial count results both after day one and day ten were significantly ($p<0.05$) different (TABLE 5).

Table 5. One way ANOVA comparative results of samples, both after Day One and Day Ten

Sample (D1)	Sample (D 10)	Parameter	f-V	p-V
Mix	Mix	APC TCC SA YM	2.14	0.1598
A	A	APC TCC SA YM	1.77	0.2485
B	B	APC TCC SA YM	13.07	0.0117
C	C	APC TCC SA YM	2.32	0.1802

D1: after Day One, D10: after Day Ten; APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeasts and Molds; f-V: calculated value; p-V: probability value.

Comparing the microbial results both after Day One and Day Ten of processing ice cream by using paired T-test, the results revealed that all the samples, in all parameters, were not significantly ($p>0.05$) different (TABLE 6).

Table 6. Paired T-test comparative results of samples, both after Day One and Day Ten

Sample (D1)	Sample (D 10)	Parameter	p-V
Mix	Mix	APC	0.3595
		TCC	0.3751
		SA	0.6943
		YM	0.3735
A	A	APC	0.2764
		TCC	0.3760
		SA	0.7780
		YM	0.3573
B	B	APC	0.4209
		TCC	0.7264
		SA	0.3739
		YM	0.1949
C	C	APC	0.3859
		TCC	0.2262
		SA	0.4096
		YM	0.3755

D1: after Day One, D10: after Day Ten; APC: Aerobic Plate Counts; TCC: Total Colony Counts; SA: Staphilococcus aureus, YM: Yeasts and Molds; f-V: Tabulated value; p-V: probability value.

IV. Discussion

The lower viability of microbial counts in ice cream samples than in the mix, both after Day One and Day Ten of processing was probably due to the freezing process and due to the amount of air incorporated [8, 9]. The declining number of viable microbial counts in ice cream products is due to freezing, most likely due to the freeze injury of cells leading to eventual death of the cells, and may be exacerbated by the incorporation of air into the ice cream [10]. The further decrease of viable counts after Day Ten was probably caused by a prolonged exposure to very low temperatures [11].

The oxidative stress probably affected the viability of microorganisms in ice cream samples under this study, proportionally to the amount of air incorporated, which may explain the lower microbial counts in ice cream samples at higher overrun levels. This is in compliance with the findings which revealed that the stress posed during the exposure to frozen storage plus the initial stress caused by the incorporation of increasing amounts of oxygen as well as the mixing step applied during the ice cream processing negatively affect the viability of microorganisms [12]. For instance, most of the studies covering oxidative stress in dairy products have emphasized the high sensitivity of *Bifidobacterium* strains to oxygen [13, 14]. Lower viability of bacteria is a result from the damage caused to the cells by the combination of freezing, and the mechanical stresses generated by mixing and incorporating oxygen into the mixture, during the manufacture of ice creams [15]. Excessive rotational speed and mixing time may lead to higher physical shearing and stress, which would disrupt the bacteria cells, impacting overall viability [16].

The presence of microorganisms in frozen dairy products is an indication of post-pasteurization contamination, which can occur from poorly cleaned equipment, air incorporation, and personnel [17]. The addition of flavors, coloring agents, and ingredients such as fruits, nuts, and chocolate chips to the mix after pasteurization can also be a source of contamination [8]. Bacteria recontaminating pasteurized milk and milk products originate primarily from water and air in the filling equipment or immediate surroundings and can be resident for prolonged periods of time [18].

In comparison of the present results with the others' findings, lower or higher incidence rates of Aerobic Plate Counts have been reported. In past studies carried out in Denizli,Turkey, low levels of APCs in ice cream were reported in the range of $1.7x10^4$-$1.7x10^5$ cfu/ml [19]. Unsatisfactory levels of Aerobic Plate Counts have also been reported in many other countries [20, 21, 22]. Many reports dealing with the occurrence of coliforms in ice cream reported coli forms counts which did not comply with the Turkish Food Codex (TFC) standard in Turkey, with detection rate of 96 % and 88.4 % [19, 23]. Unsatisfactory rates of coliforms were also reported in some developing countries, such as Pakistan and India, as 66% and 100% by [20; 22] respectively.

Lower or higher incidence rates of *Staphylococcus aureus* than in this study have been reported. In the study carried out in Turkey on ice cream, samples responded positive for *S. aureus* ranging from 10^2 to 10^4 cfu/g, and 15 % of those samples did not comply with the TFC standard of 10^2 cfu/ml [23]. Like the previous micro-organisms, unsatisfactory numbers of yeasts and molds were also reported in previous studies [22].

V. Conclusion

In this study of determining the effect of different overrun levels on microbial safety of ice cream, the findings lead to conclude that all the ice cream mixes and ice cream samples had microbial contamination most of which were above the recommended levels, indicating the post-pasteurization contamination. The different overrun levels applied in the ice cream manufacture did not significantly influence the number of bacteria counts under study. The higher overrun levels lead to increased death of bacteria in the ice cream samples during storage. However, the frozen storage period did not have a significant effect on the death of bacteria. Medium overrun levels together with good manufacturing practices should be emphasized during ice cream making.

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