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The correlation between aerobic mesophilic microorganism counts and Dornic acidity in expressed human breastmilk

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Abstract

Objective: The study was designed to test for the existence of a correlation between the total population of aerobic mesophilic microorganisms in processed raw breastmilk from a human milk bank and the Dornic acidity of that milk.

Methods: Two hundred consecutive samples of thawed expressed human breastmilk obtained from human milk bank, prior to pasteurization. Dornic acidity was titrated in triplicate for each sample. aerobic mesophilic microorganisms were then plate counted. Data were analyzed to detect correlations between variables, using Pearson's coefficient, and the level of significance was set at $p \leq 0.05$.

Results: In the samples analyzed, Dornic acidity levels had a positive ($R = 0.948$) and statistically significant ($p \leq 0.001$) correlation with the population of aerobic mesophilic microorganisms (CFU/mL).

Conclusions: The data obtained here support to the conclusion that Dornic titration is an effective method for the indirect evaluation of bacterial growth in expressed human milk.

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Introduction

Raw human milk is a food that is rich in both nutrients[1] and protective factors.[2] Its quality can be assessed in terms of nutritional, immunological, microbiological and physical and chemical properties, with physical and chemical stability having the greatest importance for the maintenance of the milk's characteristics.[3] Among those factors that affect this stability, the role of acidity has been widely discussed.[3-5]

Some of the causes that have been reported as being responsible for increased quantities of microorganisms in expressed human milk (EHM), inadequate collection techniques, donors with poor personal hygiene, unhygienic utensils and milk cold chain failure. Bacterial growth causes fermentation and acidifies the milk, and can lead to a reduction in nutritional and immunological components making the milk improper for use.[4] Acidified EHM may not meet the specific nutritional requirements of premature, underweight, or immunologically vulnerable newborn infants. Acidity destabilizes soluble proteins casein particles, promotes coagulation, increases osmolarity, alters the flavor (taste and odor) and reduces the immunological value. In carbohydrates, which are the

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bacteria's energy source, are transformed into lactic acid, which is ionized in an aqueous medium, liberating protons (H+), destabilizing casein and making calcium and phosphorus unavailable.[4] Therefore, the greater the production of lactic acid, the lower the bioavailability of calcium and phosphorus in the milk.[5]

The acidity of human milk may be an original property of the milk, determined by its constituents, or may have developed as a result of lactic acid production as lactose is degraded. this distinction does not have practical importance, since the objective is to discover the total acidity of the product.[5] Immediately after the expression, human milk is practically free of lactic acid, and its total acidity can be considered original. It is an environment favorable to the growth of microbes that makes possible lactic acid production and the progressive elevation of Dornic acidity (DA).[5]

Determination of DA is an obligatory component of the quality control at human milk banks in Brazil. The analysis aims to guarantee that the physical and chemical properties of the raw milk are maintained and represents an important element in the pre-pasteurization selection process.[4,5]

Considering the relevance of the subject, the objective of this study was to determine the relationship between the total population of aerobic mesophilic microorganisms, which represents the majority of the components of the microbes present,[6] and DA levels in EHM.

Methods

Breastmilk samples were obtained during home visits to regular donors registered with the Human Milk Bank (HMB) at the Instituto Fernandes Figueira, Fundação Oswaldo Cruz. Inclusion criteria were: expressed human milk that was mature, raw, had not been expressed specifically for that donor's own child, and that had been received frozen at the HMB-IFF in borosilicate glass flasks with perfectly sealed plastic stoppers containing at least 350 mL. The study sample comprised all qualifying milk received for pasteurization at the HMB-IFF during the period 10/05/2006 to 31/07/2006. A total of 200 samples were collected consecutively from thawed milk and once more decanted into sterile flasks during the pre-pasteurization selection process.

The study was approved by the Research Ethics Committee at the Instituto Fernandes Figueira, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil.

The parameter used for acidity control in EHM is the measurement of titratable acidity, expressed in degrees Dornic (ºD). The National Human Milk Bank Network (NHMBN) recommends that EHM with a DA above 8 ºD should be considered improper for consumption and disposed of before processing.[5]

The number of degrees Dornic were determined by the same professional for all samples, as described by Silva & Almeida,[7] titrating the milk with NaOH N/9 (Dornic solution) in the presence of a phenolphthalein indicator. Each 0.01 mL of Dornic solution expended to neutralize 1 mL of EHM is equivalent to 1º D.

After DA titration, plate counting was performed to determine the number of viable aerobic mesophile microorganisms, as described in the Compendium of Methods for the Microbiological Examination of Foods.[8] Each sample was seeded in duplicate on plates containing culture medium (Agar Plate Count, Oxoid brand) and incubated at 32 ºC. Once 48 hours had passed, colony-forming units colony-forming units (CFU/mL) were counted .

Pearson's correlation coefficient was used to test for a correlation between DA levels and mesophile populations, expressed in base ten logarithms, using the statistical package SPSS version 12 for Windows. The level of significance was set at $p \leq 0.05$.

Results

The results of data analysis are presented in Table 1. One hundred and ninety-two (96%) of the total of 200 samples analyzed were within limits approved for consumption (DA ≤ 8 ºD). The maximum CFU/mL value among these samples was 6.7×10^5. The number of CFU/mL exhibited a positive association with DA values. Each interval that DA increased corresponded to a significantly larger bacterial population. The correlation coefficient for the two variables was R = 0.948 for all 200 samples and R = 0.959 for the 192 samples with acidity less than or equal to 8 °D and therefore approved for human consumption. The correlation between the two variables was significant ($p < 0.001$) in both cases (Figure 1).

Discussion

Although EHM contains many protective substances,[2] this fact does not protect it from contamination and bacterial growth, and it can act as a vehicle for pathogenic microorganisms.[9] The conditions necessary to obtain good quality milk are well-established: control of hygienic and sanitation during collection and manipulation and maintaining the milk at low temperatures during all phases of processing, up to pasteurization and storage.[10]

Silva & Almeida[11] found high microbe counts in EHM, possibly due to external contamination. Their plate counts of mesophiles demonstrated that, for 170 samples, the bacterial population varied from 10^1 to 10^7 CFU/mL, with

the most often observed counts being in the range 10^2 to 10^3 CFU/mL. Similarly, our data varied from 10^2 to 10^7 CFU/mL with the most observed values being between 10^4 and 10^5.

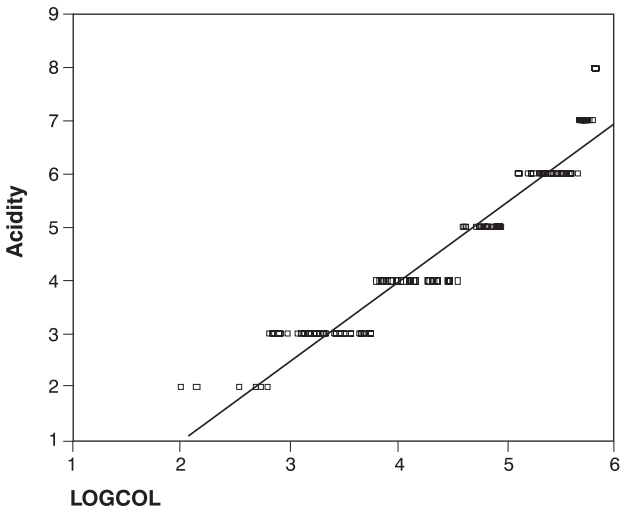
The distribution of DA observed by Silva & Almeida[12] in 172 samples of EHM was different to that observed here, although both studies identified a similar percentage of samples with acidity ≤ 8 ºD: 94.6 and 96%, respectively.

The literature describes the development of acidity in human milk as being the result of lactic acid production as lactose is broken down by microorganisms.[10,12-14] However, just two studies have evaluated this relationship.[13,14]

Silva & Almeida[13] analyzed the relationship between DA and bacterial growth in raw EHM at 37 °C. Their results revealed that, after 4 hours, the growth of mesophiles in mature milk had allowed acidity to rise progressively. In samples of colostrum, a significant increase in acidity only occurred after 16 hours' incubation, when bacterial growth raised it 28 times above its initial value. According to the authors, the difference is possibly the result of greater quantities of protective factors in colostrum. The study concluded that, in addition to rigorous hygiene, the degree of contamination of the product suffers a direct influence from its protective factors. This difference could not be

Table 1 - Distribution of frequencies in the samples analyzed (n = 200)

Dornic acidity	CFU/mL range	No. samples	Percentage per sample	Running total (%)
2	1.0×10^2 - 6.3×10^2	6	3	3
3	6.6×10^2 - 5.7×10^3	47	23.5	26.5
4	6.4×10^3 - 3.6×10^4	40	20	46.5
5	4.0×10^4 - 9.0×10^4	37	18.5	65.5
6	1.3×10^5 - 4.6×10^5	37	18.5	83.5
7	4.9×10^5 - 6.2×10^5	20	10	93.5
8	6.6×10^5 - 6.7×10^5	5	2.5	96
9	8.0×10^5 - 9.3×10^5	2	1.0	97
10	5.5×10^6 - 7.6×10^6	2	1.0	98
11	1.2×10^7 - 1.3×10^7	3	1.5	99.5
12	1.5×10^7	1	0.5	100



Pearson's Correlation Coefficient ($p < 0.001$).
CFU = colony-forming units; LOGCOL = colony-forming units (CFU/mL) logarithms.

Figure 1 - Correlation between CFU/mL and Dornic acidity for approved samples - Dornic acidity ≤ 8 ºD

analyzed in our study since, although we did use raw samples, we only analyzed samples of mature EHM.

Bortolozo et al.[14] demonstrated bacterial growth and elevation of DA in samples of frozen and pasteurized milk. Their study draws attention to the possibility of contamination and alteration of physical and chemical stability of pasteurized milk as a result of manipulation after it has been distributed for consumption.

Although the research described presents data on the correlation between bacterial population and DA in human milk, the methodological differences mean that their results cannot be compared with ours.

Luzeau et al.[15] demonstrated that fresh or pasteurized and frozen human milk can suffer lypolisis with increased DA. In acidic milks with normal lactic acid levels, alterations are the result of the increased proportion of free fatty acids, produced by lipase. Our data do not allow for the detection of other influences on the increase in DA values, such as lipid oxidation, which could have occurred.

Since the population of aerobic mesophilic microorganisms already includes the majority of contaminants present in expressed human milk, including the pathogens, and offers a general overview of the existing microbial load,[8] the fact that it maintains a strict correlation ($p \leq 0.001$) with DA values indicates that measuring DA is an effective method for indirectly assessing bacterial growth in EHM.

Further studies are important to define the role that the many different microorganisms that make up the mesophiles play in increasing the DA of EHM. Also needed is research that attempts to detect the proportion of EHM acidity caused by lipid oxidation.

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