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Sensorial analysis of expressed human milk and its microbial load

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Abstract

Objective: To verify the existence of a relationship between presence of off-flavor and microorganism load in quality control rejected samples of expressed human milk from a donor milk bank.

Methods: A total of 30 samples of expressed human milk with off-flavor were tested for the occurrence of the following microorganisms: aerobic mesophilic, psycrotrophic, proteolytic, psycrotrophic proteolytic, thermoduric, psycrotrophic thermoduric, lactate and lipolytic bacteria, molds and yeasts and *Staphylococcus aureus*, total coliforms and thermophilic coliforms, in accordance with official methods.

Results: Percentage occurrence of microorganisms was as follows: aerobic mesophilic = 80%; psycrotrophic = 36.7%; proteolytic = 46.7%; psycrotrophic proteolytic = 16.7%; thermoduric = 6.7%; psycrotrophic thermoduric = 0%; lactate bacteria = 50%; lipolytic = 10%; molds and yeasts = 6.7%; S. *aureus* = 30%; total coliforms = 53.3%; and thermophilic coliforms = 16.7%.

Conclusion: A consistent relationship between presence of off-flavor and elevated microorganism counting was observed in the analyzed samples. This correlation highlights the importance of off-flavor research during selection and quality control processes in human milk banks.

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Introduction

The high bioavailability of nutrients in expressed human milk (EHM) can provide an excellent culture medium for several microorganisms to grow. However, this implies break in biochemical barriers, i.e., the immune protection factors capable of preventing microbial growth. Once the protection factors are eliminated, the microorganisms find in EHM a medium for growing, generating undesirable substances.[1] Furthermore, EHM shows a large capacity for absorbing and adsorbing volatile substances. The room where the milk is expressed and/or manipulated should therefore be completely rid of any pronounced odors, since milk can absorb these odors, thus altering its original flavor. It is also advisable that donor mothers and health care professionals when manipulating EHM do not wear perfumes and cosmetics with strong scents or odors.[2]

Technically, flavor is both a physical and psychological perception of a combination of odor and taste in food. Regarding EHM, flavor can be classified as primary, based on the chloride-lactose relation, and secondary, originating from fatty acids and volatile compounds.[1] Off-flavor is the abnormal flavor characteristic on EHM as a result of its degradation or contamination with exogenous substances, imparting undesirable odors due to loss in product quality.[3]

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Off-flavor detection in EHM during the selection and classification processes in human milk banks (HMB) is an efficient means of quickly and safely detecting the occurrence of physicochemical modifications, such as rancidification, proteolysis and fermentation of lactose, and fixation of volatile substances.[1]

Off-flavor standards applied in HMB were developed to qualitatively describe the various types of decomposition which may have occurred due to faulty milk management during the collection, transport and storage processes.[1]

The objective of the present study is to analyze the occurrence of different groups of microorganisms in EHM samples with off-flavor and to complement a previous study carried out in our institute, which resulted in the incorporation of our test in Brazil´s HMB by the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA).[4] In that study (not published), several physicochemical tests identified the peculiar EHM odors related to the different types of decomposition observed. Thus, lipolysis was associated with the odor of coconut soap; proteolysis, with a fish-like smell; fermentation, with the smell of curd; and proteolysis/lipolysis, with the smell of rotten eggs.[5] It is important to note that there is no evidence in the current literature of similar studies.

Methods

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

A total of 30 raw EHM samples from different donors registered with the HMB at the Instituto Fernandes Figueira (HMB/IFF) were analyzed. Donor mothers were oriented to wash their hands with soap and water, and their breasts with drinking water only, before collecting milk by manual expression.

The study sample was determined after calculating sample size, starting from a variability pre-determined in previous studies, accepting a 0.5% margin of error and a t score factor that corresponds to 15 degrees of freedom and 5% of probability, employing the technique described by Leite.[6] According to this calculation, the minimum sample size required for our study was 26 milk samples.

After collection, the flasks were stored in a frozen state in the donors´ homes for no longer than 15 days, when the milk was conveyed frozen in polyvinyl chloride (PVC) isothermal containers filled with "recyclable ice" in the proportion of three cubic units of ice per one unit of EHM.

After thawing, the products were evaluated by trained professionals, who carry out regular off-flavor research in the HMB/IFF, in accordance with the norms and procedures regulated by ANVISA. Only the samples rejected due to off-flavor occurrence were used in the present study.

The samples were immediately sent frozen to the laboratory, where microbiological analyses were performed in accordance with the Compendium of Methods for the Microbiological Examination of Foods[7] and the Manual of Methods for the Microbiological Analysis of Food (Manual de Métodos de Análise Microbiológica de Alimentos).[8]

Plate counting was performed and microorganisms were grouped as follows: aerobic mesophilic, psycrotrophic, proteolytic, psycrotrophic proteolytic, thermoduric, psycrotrophic thermoduric, lactate bacteria, lipolytic bacteria, molds and yeasts, and *S. aureus*. A most-probable-number technique (mL) was applied for researching total coliforms and thermophilic coliforms.

Results

Of the 30 samples analyzed, one presented with a strong odor of medicine with negligible microbial growth. The other samples showed vigorous growth in one or various microbial groups.

Table 1 shows the percentage of contamination with different groups of microorganisms researched in the EHM samples rejected in presence of off-flavor.

The aerobic mesophilic microorganisms occurred in a number greater than or equal to 10^4 CFU/mL and, in 24 samples, countings in the order of 10^4 to 10^8 CFU/mL were observed. Out of the total, 11 samples showed psycrotrophic microorganism counting levels that varied from 10^3 to 10^7 CFU/mL. Research on proteolytic and psycrotrophic proteolytic microorganisms demonstrated that 14/30 and 5/30, respectively, showed strains in the order of 10^3 to 10^6 CFU/mL, for both groups involved. The thermoduric and the psycrotrophic thermoduric microorganisms occurred in two samples only, values varied from 10^3 to 10^4 CFU/mL. Lactate bacteria accounted positively for 50% of the samples, 43.3% being greater than 10^6 CFU/mL. Research on lipolytic microorganisms demonstrated that 3/30 samples showed numbers that varied from 10^4 to 10^6 CFU/mL. Molds and yeasts were identified in two samples only, both showed countings in the order of 10^3 to 10^4 CFU/mL. The coagulase-positive *Staphylococcus* (*aureus*) was verified in nine samples, countings varied from 10^3 to 10^6 CFU/mL. Total coliforms were verified in 16 samples, countings varied from 10^4 to 10^6 CFU/mL. Thermophilic coliforms were detected in five samples, showing numbers that varied from 10^3 to 10^4 CFU/mL.

Discussion

Detection of aerobic mesophilic microorganisms in numbers greater than 10^4 CFU/mL in 80% of the samples demonstrates the increased degree of contamination of the expressed milk. We therefore believe that these microorganisms find in expressed milk conditions favorable to their growth due to faulty milk management.[1]

Table 1 - Percentage distribution of samples contaminated with the various microorganisms/groups according to microbial load

	Counting of microorganisms								
Microorganisms/groups	**Absence**	**10^0-10^1**	**10^1-10^2**	**10^2-10^3**	**10^3-10^4**	**10^4-10^5**	**10^5-10^6**	**10^6-10^7**	**> 10^7**
Mesophilic	3.33	0.00	0.00	0.00	0.00	10.00	26.67	13.33	46.67
Psycrotrophic	8.33	0.00	0.00	0.00	8.33	8.33	33.33	33.33	8.33
Proteolytic	6.67	0.00	0.00	0.00	6.67	26.67	20.00	26.67	13.33
Psycrotrophic proteolytic	16.67	0.00	0.00	0.00	0.00	16.67	50.00	0.00	16.67
Thermoduric	33.33	0.00	0.00	0.00	0.00	66.67	0.00	0.00	0.00
Psycrotrophic thermoduric	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lactate bacteria	6.25	0.00	0.00	0.00	6.25	31.25	37.50	12.50	6.25
Lipolytic	25.00	0.00	0.00	0.00	00.00	50.00	0.00	25.00	0.00
Molds and yeasts	25.00	0.00	0.00	0.00	00.00	75.00	0.00	0.00	0.00
Staphylococcus aureus	12.50	0.00	0.00	0.00	12.50	37.50	37.50	0.00	0.00
Total coliforms	5.88	0.00	0.00	0.00	5.88	88.24	0.00	0.00	0.00
Fecal coliforms	16.67	0.00	0.00	0.00	16.67	66.67	0.00	0.00	0.00

Positivity for psycrotrophic microorganisms in 36.7% of the samples indicates problems in low temperature storage.[9,10] Detection of proteolytic and psycrotrophic proteolytic microorganisms occurred in 46.7% and 16.7% of samples, respectively. Presence of thermoduric and psycrotrophic thermoduric microorganisms in 6.7% suggests problems in manipulation or refrigeration storage regarding these samples,[7] since these microorganisms are secondary contaminants.

Lactate bacteria occurred in 50% of the samples, indicating conditions favorable to the multiplication of these fermentation microorganisms, which reduce the product pH with consequent loss of calcium and phosphorus.[11]

Lipolytic bacteria promote the development of hydrolytic rancidity, easily noticeable in its early phase due to the strong odor of coconut soap.[1] This phenomenon occurred in 10% of the samples. Molds and yeasts occurred in two samples, reflecting inadequate hygiene practices by the donors or poor hygiene when handling instruments.[7,12,13] Contamination with *S. aureus* occurred in 30% of the samples, food poisoning being a frequent association with this microorganism.[14] Presence of total coliforms indicates poor hygiene and sanitary conditions. The occurrence of these microorganisms in 53.3% of the samples suggests faulty milk management. Detection of thermophilic coliforms in 31.2% of the samples reveals a need for better hygiene and sanitary practices by a number of donors while expressing milk.[15]

In conclusion, the present study demonstrated that the off-flavor research rejected samples were exposed to secondary contaminants, probably due to inadequate manipulation and/or storage.[1] This result ratifies the importance of the off-flavor research during selection and classification of EHM in HMB. Furthermore, proof of milk contamination in the presence of peculiar odors authorizes health care professionals to advise donor mothers who store expressed milk at home to check the presence of these odors before feeding their children, thus discarding all milk with odors characteristic of deterioration or contamination.

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