

Assessment of Dornic acidity in breast milk based on storage time in the freezer and place of expression

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ABSTRACT

Introduction. The degree of Dornic acidity (DA) is an indirect measure of milk contamination and quality. In freshly expressed milk, DA ranges between 1.0 and 4.0 (optimal). If DA is > 8.0 °D, it should be discarded.

The correlation between DA in raw breast milk from internal donors (ID) and external donors (ED) based on storage time until pasteurization was assessed.

Population and method. Retrospective, analytical study.

Results. In 13 203 samples, DA was lower in IDs as of 14 days of storage: 2.92 (95% CI: 2.69–3.15) versus 4.01 (95% CI: 3.94–4.08), with a higher proportion of DA ≤ 4 °D (88% in IDs versus 76% in EDs); odds ratio: 2.30 (95% CI: 1.25–4.24).

Conclusion. DA as of 14 days of storage was lower in IDs, with a higher prevalence of DA ≤ 4 °D. No correlation was observed between storage time and DA in ID samples.

Key words: breast milk; Dornic acidity; breast milk bank; bacterial load; preterm newborn.

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INTRODUCTION

The breast milk bank (BMB) is a facility for the collection, processing, quality control, and distribution of pasteurized donated raw breast milk (RBM),¹ preferentially administered to sick and/or preterm infants, for whom the benefits of banked milk have been demonstrated compared to formula milk.^{2,3}

The quality control of donated milk is aimed at preserving its properties and the safety of the final product.⁴ Therefore, milk collection and storage must be strictly controlled, since it can be altered by heat and the proliferation of microorganisms, particularly those that break down lactose into acidic products.⁵

Dornic acidity (DA) is an indirect measure of the degree of contamination of RBM and provides information about milk quality. Freshly expressed RBM contains practically no lactic acid, and its total acidity may range from 1.0 °D to 4.0 °D. As RBM microbiota finds favorable conditions for growth, lactic acid is produced and acidity increases accordingly. Any value in the range of 1.0 °D to 8.0 °D helps to guide the criteria for the distribution of pasteurized breast milk, since the bioavailability of calcium and phosphorus and the osmolarity of the product vary inversely with acidity.^{6,7}

The BMB of Hospital Materno Infantil Ramón Sardá (HMIRS) has been operating since 2009.

FIGURE 1. Distribution of samples for analysis

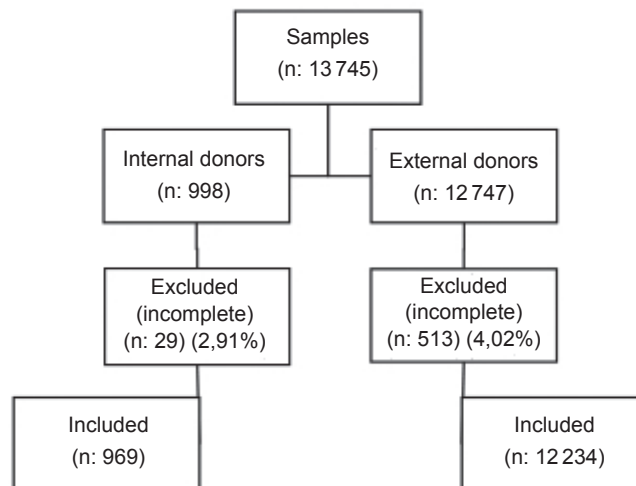
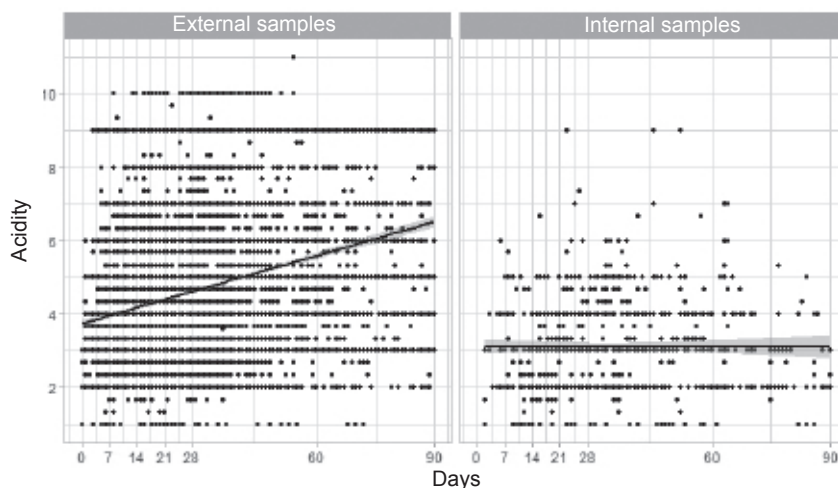


FIGURE 2. Distribution of Dornic acidity in milk samples by place of expression (external donors, n = 12234; internal donors, n = 969) and storage time



Notes: Pearson's correlation coefficient for external samples: $R^2: 0.076, p < 0.001$; internal samples: $R^2: 0, p = 1$. Intercept: external samples: 3.75 (95% CI: 3.68–3.82), $p < 0.001$; internal samples: 3.11 (95% CI: 2.96–3.26), $p < 0.001$.

Processed RBM comes from 2 different places: expressed at the donor's home (external donor, ED) or expressed at the hospitals Center for Breastfeeding (internal donor, ID). No studies have been conducted in our setting to assess the quality of donated RBM.

The objectives of this study were to assess the correlation between DA in RBM from IDs and EDs based on storage time until pasteurization and to determine the prevalence of DA ≤ 4 °D.

POPULATION AND METHODS

This was a retrospective, analytical population study about DA results in processed breast milk between 2012 and 2015 at the BMB of HMIRS on day 7, 14, 21, 28, 60, and 90 of storage and based on place of expression: samples from EDs were collected at their home, whereas those from IDs were obtained at the hospital's Center for Breastfeeding (*Supplementary material 1*).

Between 2012 and 2015, approximately 13 000 samples were collected, at a 12:1 ratio for EDs. This population allows to establish a 12% difference in the prevalence of DA ≤ 4 °D, an odds ratio (OR) of 1.60 with a 95% confidence interval (CI), and an 80% power.

Mean DA values in milk samples were analyzed using the Mann-Whitney U test. Pearson's test was used to establish the correlation coefficient (R2). A χ^2 test was done and the OR and 95% CI were described. A p value < 0.05 was considered significant. The SPSS 17® software was used.

This study complied with the law on statistical secrecy and patient confidentiality. It was assessed and approved by the Ethics and Research Committee of HMIRS (registration code: 2285).

RESULTS

Analyzed samples and DA distribution by place of expression and storage time are shown in *Figures 1* and *2*. The correlation (Pearson) between storage time and DA in samples from IDs was not significant (R2: 0, $p = 1$).

The mean DA value was observed to increase in samples from EDs in relation to storage time (*Table 1*).

A higher proportion of DA ≤ 4 °D as of 14 days of storage was observed in samples from IDs (*Table 2*).

A total of 13.90% ($n = 1701$) of samples from EDs and 0.31% ($n = 3$) of samples from IDs were discarded (≥ 8 °D).

TABLE 1. Mean Dornic acidity values by days of storage and place of milk expression (internal donors: $n = 969$, external donors: $n = 12234$)

Day	Internal donors		External donors		p
	Mean (°D)	(95% CI)	Mean (°D)	(95% CI)	
7	3.06	(2.59–3.53)	3.54	(3.45–3.62)	0.05
14	2.92	(2.69–3.15)	4.01	(3.94–4.08)	< 0.001
21	2.84	(2.70–2.98)	4.49	(4.40–4.57)	< 0.001
28	3.41	(3.22–3.61)	4.56	(4.47–4.65)	< 0.001
60	3.18	(3.06–3.30)	5.01	(4.95–5.08)	< 0.001
90	3.06	(2.83–3.29)	5.81	(5.67–5.95)	< 0.001

°D: degree of Dornic acidity; CI: confidence interval.

TABLE 2. Dornic acidity values by days of storage and place of milk expression (internal donors and external donors). Percentage over total samples with Dornic < 8 °D

Day	Internal donors n (%)		External donors n (%)		OR (95% CI)	p
	≤ 4 °D	> 4 °D and < 8 °D	≤ 4 °D	> 4 °D and < 8 °D		
7	24 (89%)	3 (11%)	728 (84%)	140 (16%)	1.54 (0.46–5.18)	0.463
14	89 (88%)	12 (12%)	1453 (76%)	451 (24%)	2.30 (1.25–4.24)	0.003
21	167 (95%)	9 (5%)	1320 (68%)	621 (32%)	8.73 (4.43–17.19)	< 0.001
28	128 (84%)	25 (16%)	1174 (67%)	582 (33%)	2.54 (1.63–3.94)	< 0.001
60	333 (83%)	66 (17%)	2088 (60%)	1379 (40%)	3.33 (2.54–4.38)	< 0.001
90	95 (86%)	15 (14%)	409 (51%)	386 (49%)	5.98 (3.41–10.49)	< 0.001

OR: odds ratio; CI: confidence interval.

DISCUSSION

According to our results, there was no increase in mean DA values for RBM samples from IDs based on the studied storage time.

After 7 days of freezer storage, there were no significant differences in DA between both milk expression sites.

After 14 days of storage, we observed significant differences in mean DA values in samples from EDs compared to IDs. A high percentage of samples from EDs (95.49%) showed DA values ≤ 8 °D, within the acceptance parameters established by the BMB and similar to those obtained by Novak et al. (96%).⁷

In relation to discarded samples, no comparison was made between donors due to the low discard percentage among ID samples. The number of discarded samples was higher among EDs based on storage time.

The bibliography, although extensive, does not strictly describe the analysis performed in our study, although there are some similarities.

Borges et al.⁹ compared samples collected at the BMB and at home preserved at -18 °C and observed no significant differences in DA values; the acceptance percentage of the samples (DA < 8 °D) was 98% and 94%, respectively. Although Borges et al. did not specify how many days had elapsed at the time of the analysis, they described that the maximum period of storage at home was 10 days, so their results were similar to those found in our study.

Vázquez et al.¹⁰ prospectively analyzed 43 samples of milk expressed at home by donors instructed in hygiene care for milk expression by the BMB and 16 samples of RBM from mothers who did not receive any advice from the BMB. In both groups, mean DA was 3 °D at time 0, prior to storage, and an increase in acidity was observed as of the first week of freezer storage, which became significant as of the second week. Mean DA values at 90 days (5 °D and 7 °D, respectively) are similar to those obtained for our samples from EDs.

Grazziotin et al.¹¹ observed DA values after 15 days of storage at -20 °C in milk expression conditions similar to those established at our Center for Breastfeeding (IDs); none of the samples showed a DA value ≥ 8 °D, and most samples ($> 80\%$) were considered top-quality milk (≤ 4 °D); these values were similar to those found in our study (88%).

Bacterial growth stops at -18 °C and there is no lactic acid production. This point may account

for some of the differences found since, during the study period, RBM expressed at the Center for Breastfeeding was refrigerated immediately after extraction, a critical point controlled by the sector staff.⁸

Although DA partly reflects bacterial action,⁷ direct analysis of bacterial growth in RBM samples by culture has not been performed, and DA values at storage time 0 should be measured. Although the intercepts (*Figure 2*) for EDs and IDs are similar to those found by Vázquez et al.,¹⁰ the difference between them could reflect a different starting point.

Lipolysis during storage is favored by storage time, increased temperature, and milk fat content.¹²⁻¹⁴

Therefore, some limitations of this study are a lack of knowledge of the initial bacterial count, the DA value at time 0, the milk fat content, and the time elapsed until the samples were kept in the freezer; these parameters may contribute to the differences observed.

It is important to carry out studies on the action of bacterial proteases and lipases involved in the breakdown of proteins and lipids of breast milk, which would contribute to a greater increase in acidic components during storage.¹⁴

The strengths of this study include its ability to assess more than 13 000 samples up to day 90, the professional work, the technique implemented in the quantification and accurate DA measurement,⁶ and, finally, the quality of the medical records during the study period.

The low Dornic acidity values and the low variability in the assessment time indicate how effective low-cost preventive processes are in obtaining and warranting safe and good-quality milk for newborn feeding in our facility.

CONCLUSION

DA values as of 14 days of storage were lower for samples from IDs, with a higher proportion of DA ≤ 4 °D. No correlation was observed between storage time and DA in samples from IDs. ■

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Supplementary material available at: https://www.sap.org.ar/docs/publicaciones/archivosarg/2023/2511_Valls_Anexo.pdf

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