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Effect of pulsed electric field on human milk processing

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Abstract
Mother's milk and breastfeeding are recognized as the natural and sustainable source for the optimal growth and development of the infants. However, when the biological mother's own milk is not available for the infants, donor human milk from human milk bank is the only alternative suggested by world health organization (WHO) and United Nations International Children's Emergency Fund (UNICEF). Holder pasteurization (62.5 °C for 30 minutes) is the widely adopted method and is currently recommended in all international mother's milk bank guidelines but it affects the bioactive components present in mother's milk. The present study was carried out using pulsed electric field (PEF) technology, which is one of the novel, non – thermal technology to process human milk samples. The effect of PEF on physicochemical properties (pH and acidity) and fat content on donor human milk before and after PEF processing were determined. No significant difference (P>0.05) was observed in pH, acidity and fat content of raw milk and PEF processed milk. High significant difference (P<0.01) in microbial reduction was observed between raw milk and PEF processed human milk samples.

Keywords: mother's milk, holder pasteurization, human milk bank, pulsed electric field

Introduction
Human milk is considered as the ideal food for the growth and development of neonates. It is composed of 87% water, 7% carbohydrate, 4% lipid and 1% protein (Boquien, 2018) [3]. The composition of the human milk is not uniform. It varies depending upon the lactation period, gestational age, breastfeeding time, genetic factors, postmenstrual age and postnatal age (Mass *et al*., 1998). Vila *et al*., (2005) [10] reported that there are three different stages in the lactational period: colostrum milk (1 – 5 days of postpartum), transitional milk (6 – 15 days of postpartum) and mature milk (after 15 days of postpartum).
Pasteurized donor human milk from milk bank is recommended by World Health Organization (WHO) when mother's own milk is not available. To serve this purpose Human Milk Bank (HMB) was established and the number of HMB's is increasing worldwide (Arslanoglu *et al*., 2013). HMB involves the process of collection, screening, pasteurization, and distribution of donated breast milk to hospitals or outpatient recipients. The most common practice followed in HMB's is to pool milk collected from multiple donors to ensure that nutrients such as protein and fat are evenly distributed (Haiden and Ziegler, 2016) [6].
Holder pasteurisation (62.5°C for 30 minutes) is widely adopted method of processing donor human milk in milk banks but this method destroys or reduces many of the unique biological properties of human milk (Modi, 2006) [9]. The preterm infants fed by pasteurized donor milk resulted in poor weight gain due to low fat and energy content in pasteurized human milk (Vieira *et al*., 2011) [12]. Pulsed electric field (PEF) technology is a novel non – thermal food preservation technique in which food to be processed is placed between two electrodes to inactivate the microorganisms and to enhance the shelf life without undergoing undesirable heat and chemical changes (Castro *et al*., 1993) [4].
The objective of this study is to determine the effect of PEF processing on fat, protein and physico – chemical parameters *viz*., pH and acidity in human milk and the ability of PEF technology in eliminating the microbial load.

Materials and Methods
Collection of samples
Human milk samples were collected from human milk banks attached to hospitals in Chennai and also from donor mother's in and around Koduveli village, Chennai. Sterilized stainless steel (SS) containers were used for mother's milk collection. pH, acidity, fat content, Total plate count (TPC) and coliform count of the collected samples were determined before subjecting the samples to PEF treatment.

To perform the analyses of the samples Automatic milk collection station was used for fat content determination, digital pH meter for pH determination and titratable dornic acidity for acidity. TPC agar and MacConkey agar were used for TPC and coliform enumeration respectively.

PEF processing of collected samples

The collected milk samples were subjected to PEF treatment using a lab model PEF equipment comprised of two stainless steel circular, parallel plate electrodes. The distance between electrodes in PEF chamber was adjusted to 0.5 cm. Pulse forming network (PFN) in PEF system produced square pulses of 2.5 µs pulse width. pH, acidity, fat and protein content of the PEF treated samples were analyzed immediately after PEF treatment. TPC and coliform were enumerated in the PEF processed human milk samples.

Statistical analysis

All the data were statistically analysed as mean ± standard deviation (SD) using SPSS software by applying one way analysis of variance (ANOVA) and Duncan multiple test.

Results and Discussion

The mean ± SE values for pH, acidity and fat content of raw and PEF processed mother's milk samples were presented in table 1.

The study revealed that there was no significant difference in pH and acidity between raw mother's milk and PEF processed milk samples. No significant difference was observed in pH and acidity of the raw milk and PEF treated samples. The pH and acidity of the PEF treated samples were >7.1 and <4˚D respectively for the raw and PEF treated samples.

Vieco *et al*., (2016) stated that donor mother's milk with a pH of ≥7.12 and acidity of ≤ 4°D was considered as top quality.

The mean ± SE values for fat content of raw mother's milk and PEF processed mother's milk samples. It was observed that there was no significant difference between the mean concentration of fat content in raw milk and PEF processed milk samples. Vieira *et al*., (2011) [12] reported a significant reduction of fat concentration by 5.5% after holder pasteurization of mother's milk. It was also observed that no significant difference in fat content between the PEF treatments. The results obtained indicates that the PEF processed milk samples were adequate for consumption by newborns, with no alteration in the fat content.

The results revealed that PEF treatment does not significantly affect the pH, acidity and fat content of the mother's milk.

Table 1: pH, acidity and fat content of raw milk and PEF processed human milk

Treatments	Ph (Mean ± SE)	Acidity (Mean ± SE)	Fat (Mean ± SE)
T_1 (25 kV, 3000 µs)	7.08±0.02	2.83±0.17	3.30±0.04
T_2 (25 kV, 4500 µs)	7.18±0.05	3.00±0.26	3.35±0.04
T_3 (30 kV 3000 µs)	7.10±0.00	2.83±0.31	3.35±0.07
T_4 (30 kV, 4500 µs)	7.17±0.04	3.17±0.31	3.45±0.06
T_5 (35 kV, 3000 µs)	7.08±0.04	2.67±0.21	3.30±0.08
T_6 (35 kV, 4500 µs)	7.13±0.07	3.17±0.31	3.48±0.05
Raw mother's millk	7.13±0.02	3.00±0.26	3.38±0.05
F-value	0.950^{NS}	0.492^{NS}	1.536^{NS}

@ average of six trials
NS – Non Significant (*P*>0.05)

Table 2 shows mean ± SE values for TPC and coliforms present in mother's milk samples before and after PEF processing. It was observed that mother's milk samples subjected to treatment T_5 and T_6 was found to be effective in eliminating the microbial load. It was observed that *E. Coli* and aerobic organisms were absent in these treatments. This is in accordance with the human milk banking guidelines which states that, in processed donor milk, no microbial growth (pathogenic or non-pathogenic) is acceptable. Any bacterial growth in the processed sample is discarded (Hartmann *et al*., 2007; Bharadva *et al*., 2014) [7, 2]. The data indicated that there was no growth in PEF treated mother's milk samples signifying PEF as an effective processing method.

Table 2: TPC and Coliform of raw milk and PEF processed human milk

Treatments	TPC (Mean ± SE)	Coliform (Mean ± SE)
T_1 (25 kV, 3000µs)	$5.86±0.01^{e}$	$1.30±0.11^{b}$
T_2 (25 kV, 4500µs)	$5.54±0.02^{d}$	$0±0^{a}$
T_3 (30 kV, 3000µs)	$5.07±0.02^{c}$	$0±0^{a}$
T_4 (30 kV, 4500µs)	$4.28±0.10^{b}$	$0±0^{a}$
T_5 (35 kV, 3000µs)	$0±0^{a}$	$0±0^{a}$
T_6 (35 kV, 4500µs)	$0±0^{a}$	$0±0^{a}$
Raw mother's milk	$6.91±0.01^{f}$	$1.89±0.45^{b}$
F- value	4872.084^{**}	307.378^{**}

@ average of six trials
NS – Non Significant (*P*>0.05)
** Highly significant (P<0.01)
* Significant (*P*<0.05)
Mean Bearing similar superscripts do not differ significantly

Conclusion

PEF treatment can be used as an alternate method for processing mother's milk. PEF treatment is effective in eliminating microbial load with no significant changes in fat content, pH and acidity.

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