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# Effect of Pulsed Electric Field on Physicochemical Parameters and Nutrient Content of Mother's Milk

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**ABSTRACT: Mother's own milk is considered as the first choice for newborns when mother's own milk is unavailable; donor mother's milk from human milk bank can be used as an alternative. Holder pasteurisation method is frequently utilised in human milk bank. During holder pasteurisation macronutrients present in the milk are not fully retained due to thermal deterioration. Pulsed electric field (PEF) a novel non thermal processing can be as an alternative method to process the donor mother's milk. In the present study the mother's milk samples were exposed to high voltage gradient of 25kV/cm and 30kV/cm by varying the number of pulses and treatment time. pH, dornic acidity, fat, protein, lactose and ascorbic acid content were analysed immediately after PEF processing and compared with holder pasteurized and raw mother's milk. When raw mother's milk samples compared with PEF processed mother's milk and holder pasteurized mother's milk it was found that there was no noticeable difference in pH, dornic acidity and macronutrients (except protein) immediately after processing. When holder pasteurised mother's milk samples compared to PEF processed samples showed highly significant difference in ascorbic acid level. Holder pasteurised milk retained only 43% of ascorbic acid whereas, PEF processed mother's milk retained 90% of ascorbic acid content. Thus, PEF processing maintains the quality of milk without compromising the nutritional content of mother's milk.**

**Keywords:** Holder pasteurization, Pulsed electric field, ascorbic acid, mother's milk**.**

## INTRODUCTION

Human milk banks facilitate the collection, processing and storage of the mother's milk from the donor mothers. When newborns cannot be fed with their own mother's milk due to preterm birth or low milk volume, in such cases donor mother's milk is the best alternative since it contains immunological components which are not found in commercial infant formula (Wight *et al.*, 2001). Holder pasteurisation method ensures the microbial safety of the mother's milk which is most often method used in human milk bank. Holder pasteurization involves heating milk at a low temperature (62.5°C) for an extended period of time (30 min). During holder pasteurisation immunoglobulin and macronutrients present in the milk are not fully retained due to thermal degradation (Buffin *et al*., 2018).

To overcome these thermal degradation technologists are looking for new alternatives to traditional thermal processing to minimize the negative effects of heat on food quality (Aguirre *et al.*, 2011). Some of the emerging innovative technologies such as high hydrostatic pressure, pulsed electric field (PEF), ultrasound, irradiation or ultraviolet are widely used to meet the increasing consumer demands for better retention of the nutritional value, sensory attributes, and longer shelf stability of processed foods such as milk, beverages (Chugh *et al*., 2014).

In recent years there is a considerable interest in pulsed electric field technology. Pulsed electric fields (PEF) is one of the most enticing developing non thermal technologies due to its short treatment times and less heating effects compared to other methods. PEF is a non thermal food preservation technique that involves the discharge of high voltage electric pulses 10-80kV/cm into a food product held between two electrodes for a few microseconds (Sujatha *et al*., 2021). In liquid media, a sequence of short, high-voltage pulses breaches the cell membranes of vegetative bacteria by expanding or forming new pores (electroporation). Pore creation can be reversible or irreversible, depending on factors such as the intensity of the electric field, the duration of the pulses, and the number of pulses (Keerthi *et al*., 2013)

This present study was envisaged to process the mother's milk using PEF technology by varying the voltage, number of pulses and treatment time and to assess the pH, dornic acidity, fat, protein and lactose content immediately after PEF processing.

## MATERIALS AND METHOD

*A. Collection of mother's milk*

The mother's milk was collected from the donor mothers in and around Koduveli village and from human milk bank attached to Institute of child health and Hospital for children. The mother's milk from the donors were collected in sterile environment in stainless steel containers and stored at refrigerated temperature until PEF processing. The HoP milk and raw mother's milk was used as control samples.

*B. PEF processing of mother's milk*

PEF processing system is composed of PEF treatment chambers and pulse generator. Coaxial PEF treatment chambers are currently widely used due to their simplicity in structure. The coaxial chamber with the capacity of 200mL was used to process mother's milk. The chamber was made up of acrylic material and SS 304 stainless steel rings (electrodes). The base of the chamber is made up of acrylic material and two hollow stainless steel rings (electrodes) was placed concentrically over the base. The distance between two concentric hollow rings was fixed to 1cm for processing 200mL of milk sample by applying a voltage gradient. PEF system consists of Pulse forming network (PFN) which produced square pulses of 2.5 µs pulse width. Different voltage gradients (25kV/cm and 30kV/cm) were applied to the milk samples at different treatment time and different number of pulses. The milk was subjected to different voltage, number of pulses and time combination as given below.

| Treatment parameters | Treatments |
|---|---|
| 25 kV, 1200 pulses 3000 µs | $T_1$ |
| 25 kV, 1500 pulses 3750 µs | $T_2$ |
| 25 kV 1800 pulses 4500 µs | $T_3$ |
| 30 kV, 1200 pulses 3000 µs | $T_4$ |
| 30 kV, 1500 pulses 3750 µs | $T_5$ |
| 30 kV, 1800 pulses 4500 µs | $T_6$ |

After PEF processing the mother's milk samples were collected in sterile condition using storage vials physicochemical parameters (pH, acidity) and nutrient content (fat, protein lactose content and ascorbic acid content) were analysed immediately after PEF processing. The PEF processed milk samples were compared with holder pasteurized and raw mother's milk samples.

*C. Determination physicochemical parameters of mother's milk*

pH was determined with a digital pH meter at room temperature. The pH meter was calibrated using pH buffer of 4.0, 7.0 and 9.2. The dornic acidity in mother's milk was estimated by titration method using 0.1N NaOH solution and 1 % phenolphthalein as an indicator. The titratable acidity was expressed in Dornic degrees (˚ D). The end point was determined by change in color from white to pale pink. Each 1mL of 0.1N NaOH required for the sample to change color accounts for 1˚ of Dornic acidity (˚D) (Sanchaya *et al*., 2021).

*D. Determination of macronutrients and micronutrients of mother's milk*

The nutrient content (Fat, protein, lactose, ascorbic acid) in mother's milk was determined using kjedhal method, Gerber method, titration with Fehling's solutions (A and B) method (Sanchaya *et al*., 2021).

*E. Statistical Analysis*

Statistical analysis was performed using SPSS and the descriptive data were reported as mean and standard error.

## RESULT AND DISCUSSION

*A. Physicochemical parameters of raw, holder pasteurized and PEF processed mother's milk*

Physicochemical parameters such as pH and dornic acidity of holder pasteurized and PEF processed mother's milk samples is shown in table 1. It was observed that there was no significant difference in pH and dornic acidity immediately after Holder pasteurization and in all treatments of PEF processing compared to raw mother's milk. Similar results were obtained by Sanchaya *et al*. (2021) who observed no change in pH and dornic acidity immediately after PEF processing. Dornic acidity did not differ significantly immediately after holder pasteurization (Roman *et al*., 2016).

**Table 1: Physicochemical Parameters of raw, holder pasteurized and PEF processed mother's milk (mean±SE)@**

| Treatment | pH | Dornic Acidity (D) |
|---|---|---|
| **$T_1$** | 7.08±0.03 | 2.33±0.17 |
| **$T_2$** | 7.08±0.04 | 2.37±0.21 |
| **$T_3$** | 7.08±0.03 | 2.33±0.22 |
| **$T_4$** | 7.07±0.03 | 2.33±0.21 |
| **$T_5$** | 7.07±0.02 | 2.37±0.21 |
| **$T_6$** | 7.06±0.03 | 2.30±0.22 |
| **HOP** | 7.07±0.02 | 2.30±0.22 |
| **Raw** | 7.06±0.03 | 2.37±0.21 |
| **F value** | $0.08^{NS}$ | $0.54^{NS}$ |

@ average of six trials (Different superscript in a same row and column differs significantly)
NS – Non Significant (P>0.05)
** Highly significant (P<0.01)
* Significant (P<0.05)
$T_1$ - 25 kV, 1200 pulses 3000 µs
$T_2$ - 25 kV, 1500 pulses 3750 µs
$T_3$ - 25 kV 1800 pulses 4500 µs
$T_4$ - 30 kV, 1200 pulses 3000 µs
$T_5$ - 30 kV, 1500 pulses 3750 µs
$T_6$ - 30 kV, 1800 pulses 4500 µs
HOP – Holder pasteurized mother's milk
Raw – Raw mother's milk

*B. Nutrient content of raw, holder pasteurized and PEF processed mother's milk*

The changes in macronutrient and micronutrients of PEF processed and holder pasteurized mother's milk samples compared to raw mother's milk is summarized in Table 2. When comparing pasteurised mother's milk to raw mother's milk immediately after PEF treatment ($T_1$-$T_6$) no appreciable variation in fat or lactose content was seen. This outcome is consistent with that of (Pitino *et al*., 2018) who found that there was no discernible difference in the amount of fat and protein present immediately after UV-C irradiation (250 nm, 25 min) and HHP (500 MPa, 8 min). After holding pasteurisation of mother's milk in comparison with raw mother's milk, no significant variation in carbohydrate content and a significant difference in fat content were found (Kim *et al*., 2022). Table 2 shows that there was a substantial difference in protein content between raw mother's milk and milk that had undergone PEF treatment and holder pasteurisation. Sanchaya *et al*., 2020 who found a substantial variation in protein immediately after PEF processing reported similar findings. The decrease in protein concentration during PEF processing may be caused by milk materials electrodepositing in the electrodes. Proteins may potentially unfurl or be oriented in the direction of the applied electric field during PEF processing which would result in a decrease in protein content (Sharma *et al*., 2014).

**Table 2: Nutrients content of raw, holder pasteurized and PEF processed mother's milk (mean±SE)@.**

| Treatment | Fat (%) | Lactose (g/100mL) | Protein (g/100mL) | Ascorbic acid (g/100mL) |
|---|---|---|---|---|
| **$T_1$** | 3.28±0.14 | 6.43±0.03 | $1.89±0.07^a$ | $4.40±0.25^b$ |
| **$T_2$** | 3.27±0.13 | 6.40±0.04 | $1.91±0.06^a$ | $4.20±0.27^b$ |
| **$T_3$** | 3.28±0.13 | 6.41±0.04 | $1.86±0.05^a$ | $4.00±0.25^b$ |
| **$T_4$** | 3.27±0.12 | 6.43±0.05 | $1.84±0.08^a$ | $4.00±0.25^b$ |
| **$T_5$** | 3.28±0.14 | 6.42±0.04 | $1.89±0.07^a$ | $4.40±0.25^b$ |
| **$T_6$** | 3.27±0.14 | 6.41±0.04 | $1.89±0.07^a$ | $4.40±0.25^b$ |
| **HOP milk** | 3.26±0.13 | 6.44±0.04 | $1.83±0.07^a$ | $2.00±0.25^a$ |
| **Raw milk** | 3.28±0.14 | 6.45±0.03 | $2.10±0.02^b$ | $4.60±0.20^b$ |
| **F value** | $0.11^{NS}$ | $0.22^{NS}$ | 2.14* | 13.12** |

@ average of six trials (Different superscript in a same row and column differs significantly)
NS – Non Significant (P>0.05)
** Highly significant (P<0.01)
* Significant (P<0.05)
$T_1$ - 25 kV, 1200 pulses 3000 µs
$T_2$ - 25 kV, 1500 pulses 3750 µs
$T_3$ - 25 kV 1800 pulses 4500 µs
$T_4$ - 30 kV, 1200 pulses 3000 µs
$T_5$ - 30 kV, 1500 pulses 3750 µs
$T_6$ - 30 kV, 1800 pulses 4500 µs
HOP – Holder pasteurized mother's milk
Raw – Raw mother's milk
(P<0.05)

The ascorbic acid content in the PEF processed mother's milk is shown in Table 2. It was observed that there was highly significant difference in ascorbic acid content in holder pasteurized mother's milk compared to raw and PEF processed mother's milk. Fast heat pasteurisation (100°C 5 mins) and slow heat pasteurisation (62.5°C 30 mins) both resulted in reduction of ascorbic acid in breast milk about 29 and 41%, respectively. Because ascorbic acid is heat-sensitive thermal pasteurisation has caused ascorbic acid to change into dehydroascorbic acid which has resulted in a drop in ascorbic acid levels (Nadal *et al*., 2008).

## CONCLUSION

In the current study no discernible variation in any physicochemical parameters and macronutrients with the exception of protein was observed immediately after PEF processing and holder pasteurization compared to raw mother's milk. In contrast, holder pasteurised mother's milk had a highly significant difference in ascorbic acid level when compared to PEF processed samples and raw mother's samples in micronutrient analysis. Because ascorbic acid is unstable to heat holder pasteurised mother's milk has retained 43% ascorbic acid than unpasteurized milk. Therefore, mother's milk samples can be processed using a pulsed electric system without compromising the nutritional content of mother's milk's.

## FUTURE SCOPE

Pulsed electric field technology can be used as an alternative to process the donor mother's milk since it preserves the nutritional quality of the mother's milk. Limitations of this study is cost of the high voltage equipment is very high. Study on immunological parameters of the pulsed electric processed mother's milk samples can be carried out in future studies.

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**Conflict of Interest.** None.

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