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**Afsaneh Dehghan,** *Shiraz University*
**Jalal Jamalian,** *Shiraz University*
**Asgar Farahnaky,** *Shiraz University*
**Gholamreza Mesbahi,** *Shiraz University*
**Marzieh Moosavi-Nasab,** *Shiraz University*

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# The Effect of Microwave Pasteurization on Some Physical and Chemical Characteristics of Milk

Afsaneh Dehghan, Jalal Jamalian, Asgar Farahnaky, Gholamreza Mesbahi, and Marzieh Moosavi-Nasab

## Abstract

In this research, cow's milk was pasteurized using microwave (MW) or HTST methods and their effects on some milk components were studied. The results showed no differences between some physico-chemical characteristics like protein, fat, acidity, and solubility percentages due to heat treatments with either MW or HTST and the control samples. The contents of six amino acids (aspartic acid, glycine, glutamic acid, histidine, arginine, and lysine in mg/L) and fatty acids (weight percentage) showed no significant differences using either MW or HTST pasteurization method. There were slight but insignificant differences in trans fatty acid and D-amino acid contents in the milk pasteurized with either MW or HTST method. SDS-PAGE and HPLC analysis of milk proteins did not reveal any differences between the pasteurization methods. It was finally concluded that MW heating is a good alternative to HTST pasteurization.

**KEYWORDS:** microwave, HTST, milk pasteurization, milk amino acids, milk fatty acids

**Author Notes:** Marzieh Moosavi-Nasab, Ph.D
Associate Professor of Food Science and Technology and Director of Seafood Processing Research Group
College of Agriculture, Shiraz University, Iran
Telefax: (0098711) 228-7029
Correspondence for this manuscript can be addressed to:
Asgar Farahnaky, Ph.D
Associate Prof of Department of Food Science and Technology
School of Agriculture
Shiraz University, Iran
email: farahnak@shirazu.ac.ir Tel: 0098-711-6138229- 6138223, Fax: 0098-711-2286110

## 1.Introduction

Pasteurization is a conventional process applied to milk for destruction of pathogenic bacteria, yeasts and fungi. One of the pasteurization methods that causes retention of nutritional and sensory attributes is HTST method in which milk is pasteurized by a continuous system of plate heat exchangers at 72 °C for 15 seconds. However, this method causes sedimentation on the surface of plates which leads to a reduction of heat transfer. To keep heat-sensitive nutrients and decrease fouling, heating with MW is more appropriate. At present, pasteurization and sterilization by MW have been developed in the USA and Europe industrially. MW is a kind of non-ionized electro-magnetic energy with 300 up to 300000 MHz. The frequencies of these waves are higher than radio waves and lower than infra-red and visible waves which generate heat inside the food (Ohlsson, 2001). MW has numerous applications in food industries such as blanching, drying, cooking, pasteurization, and sterilization (Sumnu & Sahin, 2005). Some researches have been carried out related to MW application in milk pasteurization and only a few of these have been published due to economic and political reasons. Jeppson (1964) was the first to report application of continuous MW for pasteurization of defatted milk in glass jars while, Hamid et al. (1969) pasteurized milk in batch and continuous modes. The continuous system was devised using a glass tube placed obliquely across a waveguide. The results showed that MW was effective in decreasing microbial count. Pursuant to this, continuous system of MW pasteurization of milk was developed by others (Stenstrom, 1972; Jaynes, 1975; Sale, 1976; Lopez- Fandino et al., 1996).

Cross et al. (1982) reported that there is no significant difference in amino acid isomerization of milk heated with MW or HTST, but later Lubec et al. (1989) affirmed the presence of D-proline (1-2 mg/L) in MW milk.

Herzallah et al. (2005) and Veronika- Salamon et al. (2007) heated raw milk using MW and HTST methods and declared that there is no difference in the fatty acid contents of heated milk compared to the control sample. Also the content of elaidic acid increased in milk heated in either MW or HTST method with no appreciable untoward effects on human health.

While an abundance of literature supports the use of MW, research studies on MW pasteurization of milk have yielded limited and contradictory data. Hence, in this research the development of industrial MW pasteurization of milk compared to conventional methods has been investigated. For this purpose cow's milk was pasteurized using MW and HTST methods and the effects of these two processing methods on microbiological and some physico-chemical characteristics such as protein, amino acids, fat and fatty acids were compared.

## 2.Materials and methods

### *Milk samples*

Raw cow's milk was obtained from an experimental animal station (College of Agriculture, Shiraz University).

### *Microwave and conventional treatments*

MW heating was carried out using a LG MW oven (MC-789Y) with 2450 MHz and 540 W power. First, raw milk samples were homogenized (West Steel, Iran, 150 bar) and divided into 400-ml portions in 1000-ml beakers, then heated in the MW oven for 6 min until the temperature reached 85 °C and were held at this temperature for 15 seconds. Then, they were cooled in ice-water. Milk was also pasteurized in an HTST system (Alfa-Laval, Sweden) under the conditions comparable to MW (85 °C, 15 s). Raw and treated (MW and HTST) samples were transferred aseptically into 10-ml plastic tubes and stored at -80 °C for further investigations.

### *Analytical determinations*

All analytical determinations were carried out in triplicate. Adequacy of pasteurization was confirmed by phosphatase test (AOAC, 2002). Standard plate methods using standard agar and coliform counts were carried out by enumeration on violet red bile agar followed by incubation at 30 °C for 48 h. (Clare et al., 2005). Protein and fat contents of milk samples were measured by micro-Kjeldahl and Gerber methods, respectively (AOAC, 2002).

Analysis of D- and L-amino acids and also free amino acid contents, resulting from heat treatment of milk, was performed using an HPLC method (Rubio-Barroso et al., 2006). The amino acids investigated were: D- and L- Ala., D- and L-Ser., D- and L-Val. and D- and L-Trp. and the reference amino acids were supplied by Sigma Chemical Co. Standard aqueous solutions of D- and L-amino acids at concentration levels of 25 mg/L were prepared and stored in glass bottles at 4°C. An HPLC system equipped with the following components was used: a Waters 1525 Binary HPLC pump, a Symmetry C-18, 5-µm particle size column (150 × 4.6 mm) kept at 30°C, for D- and L-amino acid separations and a Phenomenex safeguard column. The mobile phase was prepared by mixing 12.5 m*M* sodium phosphate buffer solution, pH 6.5 (solvent A) with methanol/solvent A/THF (100/60/6,vol/vol/vol; solvent B). The solvent was applied with a flow rate of 1 ml/min at 30°C. A Waters 2475 multi λ fluorimetric detector was used at 344 and 443 nm excitation and emission wavelengths, respectively. The injection volume of derivatized amino acids was 20 µL (Williams, 1988).

Milk fatty acid contents including elaidic acid were analyzed by gas chromatography (Ledoux et al., 2000). The gas chromatograph used was equipped

with a flame ionization detector. Helium was used as carrier gas with a flow rate of 3.7 ml/min; a BPX70 capillary column (50 m ×0.32 mm) was kept at 70 °C for 3 min, heated at 9 °C/min until 175 °C and held for 27.5 min, then heated at 1.3 °C/min until 210 °C and held for 10 min (Wolff et al., 1998). Identification of certain fatty acids including elaidic acid was based on their retention times compared with reference standard.

Acidity of samples was measured by the standard method (AOAC, 2002). For determination of soluble protein of milk, first casein was precipitated by acidifying milk to pH 4.6 with concentrated HCl. The precipitate was separated from the whey fraction by centrifugation (4000g, 25 min., 4 °C) and then filtration. Soluble protein in whey was measured by Kjeldahl method (AOAC, 2002). Total protein of milk samples was also measured by Kjeldahl method (AOAC, 2002). Protein solubility percentage was calculated as follows:

$$\text{Protein solubility\%} = \frac{\text{Soluble protein}}{\text{Total protein}} \times 100$$

Sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) of milk samples was performed as described by Laemmli (1970). Milk proteins were separated in polyacrylamide gel (12.5%) and stained with Coomassie blue R250.

The color of milk samples was determined using optically controlled conditions by a Cannon digital camera (A710 model, Japan). A picture of each sample was taken under controlled conditions and opened in Photoshop software 9. From each sample the average of 2×2 cm area was randomly analyzed and reported as the result of colorimetry (Yam & Papadakis, 2004, Afshari-Jouybari, & Farahnaky, 2011).

Data were analyzed using SPSS software (Version 13). Significant effects of heat treatments on all parameters listed were evaluated by ANOVA with means separation.

## 3.Results and discussion

Milk pasteurization by MW or HTST was confirmed by the alkaline phosphatase test, standard plate and coliform counts. It was found that the total microbial and coliform contents reduced compared to the raw milk. The protein and fat contents of pasteurized and raw milk samples are shown in Table 1. As shown, neither the protein nor the fat contents in both pasteurization methods are significantly different ($p<5\%$) from the raw milk. This means that MW pasteurization similarly to HTST pasteurization maintains the level of both protein and fat in milk, in line with the results of Kumar et al., 2006.

Table 1. Total protein and fat contents of pasteurized and raw milks (means ± SD).

| Product | Protein (%) | Fat (%) |
|---|---|---|
| Raw milk | 3.43[a] (±0.05)[*] | 3.30[a] (±0.00) |
| MW milk | 3.42[a] (±0.05) | 3.26[a] (±0.02) |
| HTST milk | 3.46[a] (±0.02) | 3.26[a] (±0.05) |

The results of effects of MW and HTST methods on the level of six free amino acids (aspartic acid, glutamic acid, glycine, histidine, arginine and lysine) showed that there were no significant differences between the heated and raw milk samples with regard to glutamic acid, glycine and arginine, whereas the aspartic acid content of HTST milk was decreased significantly compared to raw or MW milk. Also as shown in Table 2, the lysine content in MW milk was significantly lower than those for raw or HTST milks. (0.45 mg/L against 0.70 and 0.65, respectively). The differences in the latter amino acids in MW and HTST methods could be due to experimental error or the nature of amino acids (Gandolfi et al., 1992; Buck et al., 1987; Rubio- Barroso, 2006).

Table 2. Free amino acid contents (mg/L) of pasteurized milks with MW and HTST methods (means ± SD).

| Amino acid | Product | | |
|---|---|---|---|
| | Raw milk | MW milk | HTST milk |
| Asp | 2.75[b] (±0.07)[*] | 2.60[b] (±0.00) | 1.90[a] (±0.14) |
| Glu | 39.65[a] (±2.40) | 40.65[a] (±0.35) | 37.30[a] (±2.96) |
| Gly | 8.20[a] (±0.56) | 8.20[a] (±0.70) | 8.40[a] (±0.42) |
| His | 12.65[a] (±0.49) | 13.85[a] (±0.63) | 12.70[a] (±0.56) |
| Arg | 7.00[a] (±0.42) | 7.40[a] (±0.14) | 7.35[a] (±0.77) |
| Lys | 0.70[b] (±0.00) | 0.45[a] (±0.07) | 0.65[b] (±0.07) |

The effect of heat from different pasteurization procedures on the production of D isomers of serine, alanine, valine and tryptophan is shown in Table 3. As seen, the L isomers of serine and alanine significantly decreased ($p<5\%$) in both heated milk samples compared to the raw milk, while the D forms of these amino

acids in heated samples were not significantly different from the unheated control. Hence it can not be stated that the decrease of L-amino acid is necessarily due to its conversion to the D isomer but that the L forms may have participated in Maillard reactions (Fay et al., 1991). D and L forms of valine and tryptophan were not significantly affected by any of the pasteurization methods. According to the present results, milk pasteurization with MW can not lead to production of D isomer of amino acids to any significant degree and the slight differences in amino acid isomers could be because of the nature of each amino acid (Buck et al., 1987).

Table 3. D and L isomer contents (mg/L) of serine, alanine, valine and tryptophan in pasteurized milks by MW and HTST methods (means ± SD).

| Amino acid | Product | | |
|---|---|---|---|
| | Raw milk | MW milk | HTST milk |
| D-Ser | $0.08^{a}$ (±0.03)* | $0.06^{a}$ (±0.00) | $0.06^{a}$ (±0.00) |
| L-Ser | $2.60^{b}$ (±0.14) | $1.10^{a}$ (±0.42) | $1.40^{a}$ (±0.14) |
| D-Ala | <0.04 | <0.04 | <0.04 |
| L-Ala | $4.00^{b}$ (±0.14) | $3.60^{a}$ (±0.00) | $3.55^{a}$ (±0.07) |
| D-Val | <0.06 | <0.06 | <0.06 |
| L-Val | $0.92^{a}$ (±0.08) | $0.80^{a}$ (±0.24) | $0.82^{a}$ (±0.09) |
| D-Trp | <0.06 | <0.06 | <0.06 |
| L-Trp | <0.05 | <0.05 | <0.05 |

Fatty acid contents of milk pasteurized with MW and HTST methods and the raw milk are shown in Figure 1. As shown, fatty acid contents of heated milks are not significantly different from those of raw milk. Thus, milk pasteurization with MW does not change its fatty acid content ($C_4$- $C_{18}$) and that its content is within the standard limit (Garcia – Ayuso et al., 1999; Veronika-Salamon et al., 2007).

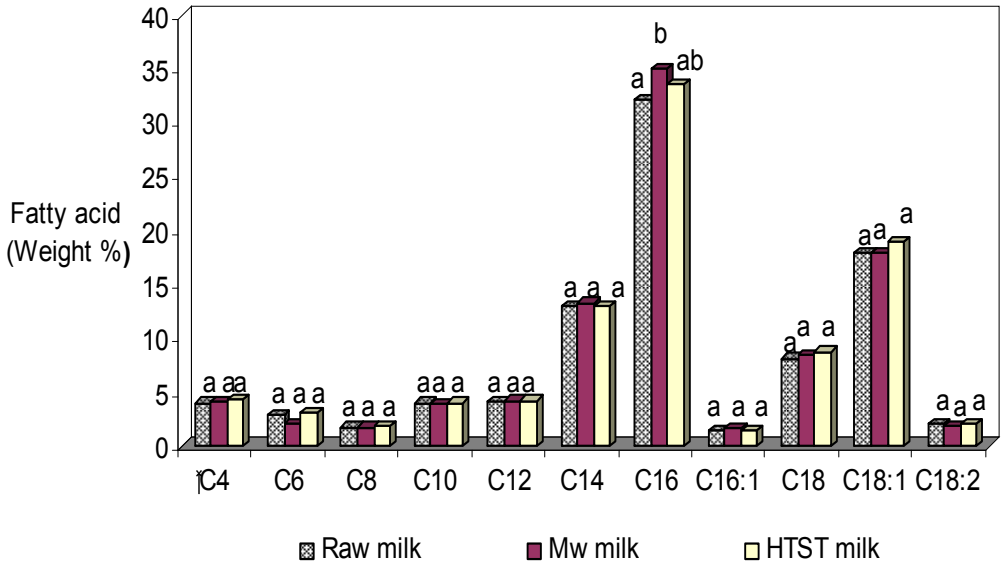


Figure 1. Effect of pasteurization with MW or HTST methods on milk fatty acids. (Bars denoted with similar letters are not significantly (P<5%) different).

The effect of heat on oleic acid (cis isomer) and elaidic acid contents (trans isomer) shows that the elaidic acid content increased compared to the control (1.45% in raw milk vs. 1.47% and 1.71% in MW milk and HTST milk, respectively). However, this increase is not significant (Table 4). Therefore, pasteurization (85 °C for 15 s) with MW or HTST does not have any significant effect on the elaidic acid content (Herzallah, 2005; Veronika-Salamon et al., 2007).

Table 4. Elaidic acid content (weight percentage, means ± SD) of milk pasteurized by MW and HTST methods.

| Product | Oleic acid | Elaidic acid |
|---|---|---|
| Raw milk | $17.96^{a}$ (±0.33)* | $1.45^{a}$ (±0.13) |
| MW milk | $17.95^{a}$ (±0.28) | $1.47^{a}$ (±0.12) |
| HTST milk | $18.90^{a}$ (±1.56) | $1.71^{a}$ (±0.06) |

No important differences were observed in the acidity values of all samples treated by MW or HTST (all being estimated at 15% dornic), similar to the results of Valero et al., 2001.

Because of heating, whey protein hydrophobic groups typically buried within the core of the protein structure are exposed to the surface and cause a decrease in protein solubility of processed samples compared to the control. This decrease was insignificantly higher in MW than in the HTST method as shown in Table 5.

Table 5. Protein solubility percentages of pasteurized and raw milks (means ± SD).

| Product | Protein solubility(%) |
|---|---|
| Raw milk | $28.45^{b}$ (±0.40)* |
| MW milk | $22.97^{a}$ (±0.48) |
| HTST milk | $24.64^{a}$ (±0.66) |

Figure 2 shows SDS-PAGE pattern on acrylamide gel (12.5%) for milk samples. Protein bands of milk samples have been identified (αs-casein, ß-casein, ß-lactoglobulin, α- lactalbumin and 79 kDa-band ) and point to the fact that the protein bands and their intensities in different treatments are very similar which is in line with the results of others (Kaddouri et al., 2006; Havea et al., 1998).

Color measurements of milks pasteurized by MW or HTST methods and also raw milk were made by digital photography and the results are reported in Table 6 as L, a, b parameters. L values in pasteurized milks significantly decreased compared to the raw milk ($p<5\%$) b values which show the yellowness of the product increased in heated milks. Heating milk leads to release of some amino acids and since temperature and pH (rather alkaline) are suitable for Maillard reaction, this reaction occurs in the heated milks and produces brown compounds, hence a decrease in L and an increase in b is observed (Clare et al., 2005; Valero, 2001 ; Contarini, 1997).

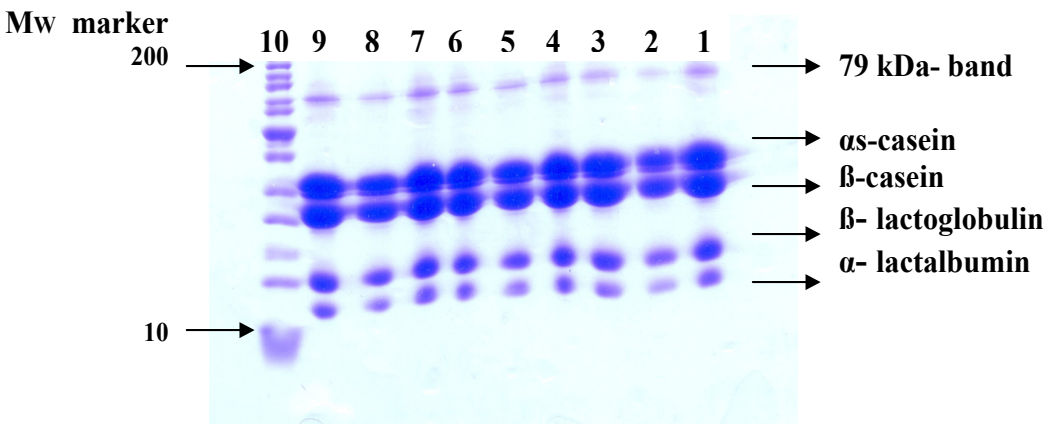


Figure 2. The SDS-PAGE pattern of the milk treatments on 12.5% gel; columns 1-3: raw milk, columns 4-6: MW milk, columns 7-9: HTST milk and column 10: molecular weight standard. The SDS-electrophoretic profile of the milk proteins contains five major bands identified as: αs-casein, ß-casein, ß-lactoglobulin, α-lactalbumin and 79 kDa-band.

Table 6. Color parameters L, a and b in milks pasteurized with MW and HTST (means ± SD).

| Product | Color parameters | | |
|---|---|---|---|
| | L | a | b |
| Raw milk | $70.67^{b}$ (±0.57)* | $4.67^{a}$ (±0.57) | $14.34^{a}$ (±0.57) |
| MW milk | $69.34^{a}$ (±0.57) | $5.00^{a}$ (±0.00) | $15.34^{b}$ (±0.57) |
| HTST milk | $69.00^{a}$ (±0.00) | $5.00^{a}$ (±0.00) | $16.00^{b}$ (±0.00) |

## 4. Conclusions

Based on the present results, milk pasteurization with MW, does not have any negative effects on nutritional value of milk (i.e protein, fat, amino acids and fatty acids) as compared to HTST method. The results obtained in this research indicated that during MW processing the milk was affected by heating mechanism similar to HTST system, and non-thermal mechanisms had little effect. Considering the shorter pasteurization period and the lower demand for energy and personnel, it can be concluded that MW pasteurization enjoys special merits in the milk pasteurization industry.

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