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The fluorimetric FAST method, a simple tool for the optimization of microwave pasteurization of milk

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The effect of microwave pasteurization of cow's milk on its nutritional quality was examined by the FAST method (Fluorescence of Advanced Maillard products and Soluble Tryptophan). Raw milk samples were submitted to different laboratory scale in batch microwave treatments using a central composite experimental design based on specific power and treatment time. The FAST index and bacterial count were monitored to assess protein denaturation, modification by the Maillard reaction and pasteurization efficiency, respectively. High discrimination between samples indicated that the FAST method is a potent tool for estimating the deterioration of the milk quality during experimental microwave treatment. Thus, the FAST index can be effectively used as the continuous response in experimental designs set up and to maximize information economically. In short, the FAST method allows us to retain the rapidity of experimental design while providing the advantages of convenience and low cost.

Keywords: FAST method / Fluorescence / Maillard reaction / Milk / Pasteurization

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1 Introduction

Heat treatment of milk is commonly used to ensure microbial safety and to supply milk with longer shelf-life. Commercial sterilization and pasteurization are the most frequent processes in the treatment of milk. The former leads, theoretically to a complete removal of bacteria and cells, whereas the latter process is more gentle and only guarantees the destruction of the pathogenic bacteria (*i.e.*, *Mycobacteria*, *Staphylococci*, and *Streptococci*) without destroying all of the harmless microorganisms and spores. According to the European council directive 92/46/EEC, pasteurized milk is defined as a milk that shows a negative reaction to the phosphatase test and a positive reaction to the peroxidase test [1]. A sample of milk can be defined as sterilized when no deterioration can be observed after it has spent 15 days in a closed container at a temperature of +30°C. The same European regulation defines the pasteurization method for milk as heating at 71.7°C for 15 s or any time and temperature combinations that provide an equivalent effect. This technique has been named flash pasteurization or high-temperature short-time pasteurization (HTST) in comparison to the original pasteurization technique which was set up at 66°C for 30 min. However, after the finding of a more resistant pathogenic micro-organism in raw milk, *Mycobacterium avium* subsp. *paratuberculosis*, the United Kingdom dairy industry has decided to increase the time for milk pasteurization from 15 to 25 s [2]. The pasteurization process now varies from one country to another according to specific national regulations.

Although the short holding time of flash pasteurization limits the thermal damage caused to milk constituents, nutritional and sensory changes may still affect the overall quality of milk. The Maillard reaction occurring in heated milk is one of the most important causes of nutritional loss [3], and the formation of off-flavors [4]. In addition, it induces the formation of yellow and brown pigments, the polymerization of milk protein as well as other chemical modifications [5]. The nutritional changes are mainly due to a significant loss (2–3% in pasteurized and 7–10% in ultra-high pasteurization (UHT) milk) of the essential amino acid lysine *via* its nonenzymatic reaction with lactose [3]. Following these, the Amadori product lactulosyllysine that is formed undergoes advanced Maillard reaction which produces stable end-products only partially identified [6].

No matter which pasteurization method is used, a compromise must be reached between completely eliminating the pathogenic bacteria and reducing the number of other spoi-

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Abbreviations: FAST, Fluorescence of Advanced Maillard products and Soluble Tryptophan; **MRPs,** Maillard reaction products; **UHT,** ultra-high pasteurization

lage organisms, and retaining the functional and organoleptic qualities of the native milk proteins.

To achieve this goal several alternative pasteurization methods are under investigation. The methods being studied, or already in use in the industry, include microwave [7], high pressure homogenization [8], nonthermal electric pasteurization [9], and bactofugation [10]. Microfiltration [11] is another alternative technology which directly eliminates bacterial contaminants by filtering the defatted milk through a 1.4 μm porous membrane. By testing the heated milk for the presence of selected bacteria technologists check the performance of a pasteurization method. The total bacteria count in a sample is recorded as the number of colony-forming, units *per* milliliter. Another indirect method has been proposed based on peroxidase activity [12]. But other chemical markers such as the early Maillard reaction product (MRP) lactulosyllysine transformed into furosine by acid hydrolysis [13] or specific whey proteins [14] have been used. Due to different heat sensitivities, none of them can be used on the full scale of temperature and time usually applied during milk heat-treatment. Furthermore, the analytical methods employed for the quantification of the heat-damage markers are complex, frequently requiring more than one step such as extraction, derivation and chromatography. Birlouez-Aragon *et al.* (1998, 2002) [15] have developed a simple, rapid, and cost-effective fluorimetric technique to estimate the intensity of heat treatment applied to milk using the physico-chemical properties of milk proteins. This method, called FAST for Fluorescence of Advanced Maillard products and Soluble Tryptophan, was initially used to discriminate milk heat-treatments. This new method seems to be suitable for a rapid on-site evaluation of the functional and nutritional damage of milk brought about by experimental pasteurization.

The aim of our study is to demonstrate the usefulness of the FAST method for the optimization of microwave pasteurization as an alternative milk pasteurization technology.

2 Materials and methods

2.1 Milk supply

Raw milk from healthy Prime Holstein cows was collected from the herd at the Institut Supérieur d'Agriculture de Beauvais. The mean fat and protein contents in the raw milk were 40.4 and 32.6 g/L, respectively. Sterilized, pasteurized, and microfiltrated commercial milks were purchased from a local supermarket.

2.2 Microwave equipment

Microwave treatment was performed on a laboratory-scale pilot plant designed by MES Technologies Company. This equipment which runs at 2450 MHz (Fig. 1) is composed of a microwave generator, a magnetron, with a power range between 0 and 1860 W, a wave guide equipped with a circulator device which allows the reflected power to veer toward a water load and finally a stereomodal® cavity, patented by MES Technologies, provided with a revolving plate intended to receive the product. In this kind of cavity, only a few known wave mode propagations are selected which leads to a better coupling between the waves and the product than in a multimodal one.

Milk samples of 250 mL were placed in a thin walled glass beaker of 500 mL. Three optical fibers were used to measure the milk temperature during the treatment. All the data were collected on a data logger (SA 70 AOIP) controlled by

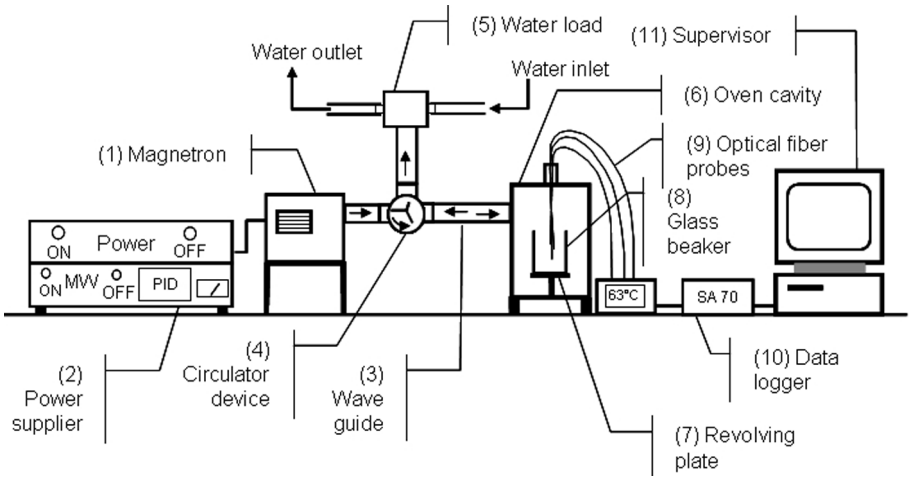

Figure 1. Scheme of the laboratory-scale microwave pilot plant.

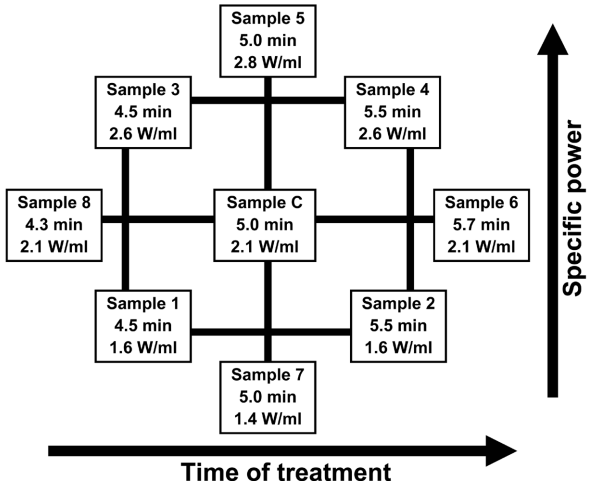

Figure 2. Experimental design of the microwave treatment of milk.

a Labview® supervisor software. At the end of the microwave treatment, the milk samples were analyzed immediately for the microbial count. Ten milliliters of each sample were also rapidly cooled and stored in a freezer at −22°C until FAST analysis was carried out.

2.3 Experimental design

A central composite design with two factors (microwave specific power and heating time) was used. Figure 2 presents the different combinations of factors selected in this study. The specific power was set up between 1.4 and 2.8 W/mL and the time of treatment from 4.3 to 5.7 min. The central combination, named C, was done in triplicate. Two responses were considered: the FAST index was used as the continuous response to be minimized, whereas microbial count was used only to check the pasteurization efficiency.

2.4 Microbial count

Enterococcus faecalis (Pasteur Institute Strain 76.117) was selected as our target micro-organism to determine the effectiveness of pasteurization. The initial culture was activated by transferring it into an Erlenmeyer flask (500 mL) containing 100 mL of Brain Heart Infusion (Difco) and incubated at 30°C for 14 h prior to inoculation of the samples.

This culture was added in the raw milk to obtain a final concentration of approximately 5×10^6 CFU/mL before microwave treatment.

At the end of each treatment the inactivation of *E. faecalis* by microwave pasteurization was measured by plate counts of the heated milk samples. Successive dilutions of the milk samples were made and stored at room temperature for 30 min to limit stress of bacteria. One tenth milliliter of each of the diluted samples was then spread in triplicate on to a Slanetz and Bartley agar (Difco) plate and incubated at 37°C for 48 h. The number of *E. faecalis* colonies that grew on each plate was counted. For the optimized point, the plating was repeated five times on Slanetz and Bartley medium to ensure the absence of *E. faecalis*. In parallel, a positive control of medium was realized to confirm its good preparation.

2.5 FAST method

Both commercial milk and samples from the experimental design were analyzed with the FAST method, as described by Birlouez-Aragon *et al.* [15] One milliliter of milk sample was added to 9 mL of sodium acetate buffer (100 mM, pH 4.6). After being shaken vigorously for 30 s the sample was centrifuged at 4000 rpm for 10 min at room temperature in a Beckman-Coulter Avanti® J-E centrifuge. The supernatant formed was filtrated through a 0.45 µm pore nylon filter (Cluzeau, France) and transferred into a disposable acryl cuvette (Sarstedt, France) that was placed in a Varian spectrofluorimeter (model Cary Eclipse) for analysis. Two emission fluorescence intensities were measured on each sample to calculate the FAST index: the fluorescence of tryptophan (F_{Trp}) at an excitation of 290 nm and an emission of 340 nm and the fluorescence of the advanced Maillard products (F_{AMP}) at an excitation of 330 nm and emission of 420 nm. The FAST index was calculated as $(F_{AMP}/F_{Trp}) \times 100$.

3 Results and discussion

The temperature evolution of each treated milk sample is shown in Fig. 3. The initial temperature of the milk was between 10.7 and 14.6°C. Depending upon the specific power and the time of microwave treatment, the end temperature was found to be between 66.9 and 102.3°C. Samples 3, 4, and 5 reached a temperature plateau that could not exceed the boiling point of milk (100 – 102°C).

After an immediate cooling of the milk in a freezer at – 22°C, the samples reached the temperature of 5°C in 16 – 19 min.

The FAST method estimates the physico-chemical modifications of heat-treated milk by measuring two different fluorescence intensities in the pH 4.6 acetate soluble fraction of the samples. In this acidic condition, most of the

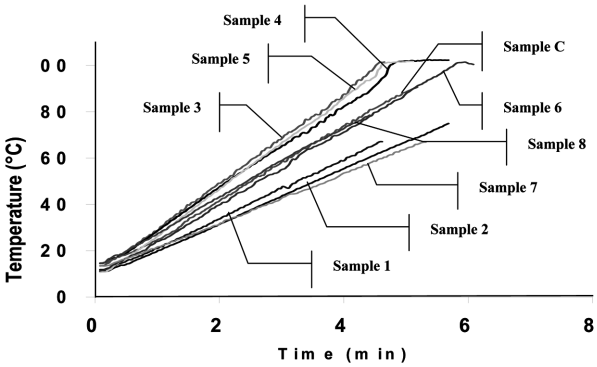

Figure 3. Evolution of milk as a function of the temperature during the microwave treatments.

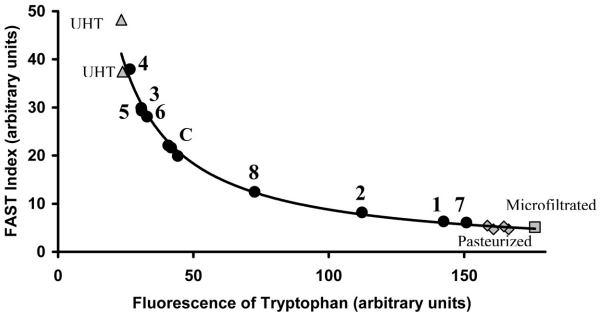

Figure 4. Relation between Tryptophan fluorescence and FAST index in commercial and experimental heat-treated milk samples. UHT samples (▲), pasteurized samples (♦), microfiltrated sample (■), and samples from the experimental design (●).

milk proteins precipitate and only the nondenaturated whey proteins remain in solution [14]. Therefore, the first fluorescence measurement (290/340 nm) estimates the extent of heat denaturation of whey proteins. By recording the intensity of tryptophan fluorescence this technique indirectly measures the soluble protein content in the acidic supernatant [15]. The second measurement (330/420 nm) estimates the quantity of MRPs in the same supernatant. Finally, the FAST index is a mathematical formula that standardizes the Maillard fluorescence as a function of the protein concentration in the acid soluble supernatant.

Birlouez-Aragon *et al.* [15] have proposed a 2-D representation that visualizes the discrimination of milk samples according to their heat treatment. This representation was selected to present the results of our study. Figure 4 shows each sample of the microwave experimental design plotted on a graph with the tryptophan fluorescence on the *X*-axis and the FAST index on the *Y*-axis. The nine samples were distributed on a single regression curve. The few commercial milk samples were also aligned along the same curve. As expected the pasteurized and microfiltrated milk samples had low FAST indices and high tryptophan fluorescence compared to the sterilized milk samples that had high FAST indices and low tryptophan fluorescence.

From the experimental design, milk samples 1, 2, and 7 treated with low specific powers (≤1.6 W/mL) had the highest Trp fluorescence and the lowest FAST index. This indicates that the microwave treatments used for theses samples limited both the protein denaturation and the formation of MRP. However, this figure also shows that these moderately microwave-treated milk samples can be discriminated easily on the Trp fluorescence axis but not so clearly on the FAST index axis.

Milk samples 3, 4, and 5 treated with the highest specific powers (≤2.6 W/mL) had the highest FAST indices among the nine samples of our experimental design, and a very low Trp fluorescence, similar to what is observed in UHT sterilized milk.

Under severe heat treatment, samples are better discriminated according to their content of advanced Maillard products and not by their protein denaturation, which seems to be almost total. Intermediate microwave treatments (samples 6, 8, and C) were associated to medium FAST responses as shown in Fig. 4.

Interestingly this study shows that during the microwave treatment, the milk proteins are first denatured and then followed by the formation of fluorescent advanced MRP. Furthermore, with an experimental design, the FAST index is a perfect indicator (continuous response) to select. The present experimental design based on the two factors, specific power and time of treatment, gave the following quadratic model:

$$\text{FAST} = -35.4 + 13.47P_s + 0.029t + 6.17P_st - 5.43P_s^2 - 0.5t^2$$

with the specific power (P_s) and the time (t) of treatment as the main effects, and their possible interaction as the third effect.

The three-dimensional plot presented in Fig. 5 shows how the response varied as a function of the two tested factors. Even though both factors had an effect, the main increase in the FAST index was induced by an increase in the microwave specific power.

It has been demonstrated that the FAST method has advantages such as sensitivity and selectivity over methods such as HPLC quantification of furosine. Additionally, it is also important to note that practical and economic considerations also provide distinct advantages to the FAST method. While an untrained technician can easily complete ten analyses using the FAST method in 20 min using a bench-top fluorimeter, analysis of furosine (one currently used index

Table 1. Microbiologic measures obtained for each specific power and time combination of the experimental design

Sample no.	Combinations specific power and time	Final temperature (°C)	F_{Trp}	FAST index	C_0 (CFU/mL)	C (CFU/mL)
1	1.6 W/mL, 4.5 min	67.3	142.4	6.29	5.3×10^5	71
2	1.6 W/mL, 5.5 min	74.9	112.2	8.16	5.3×10^5	1.0[a)]
3	2.6 W/mL, 4.5 min	101.5	30.8	29.9	5.3×10^5	0
4	2.6 W/mL, 5.5 min	102.3	26.5	37.93	5.3×10^5	4.0[a)]
C (central)	2.1 W/mL, 5 min	90.6	44.8	20.34	4.5×10^5	1.5[a)]
5	2.8 W/mL, 5 min	101.8	30.9	29.34	4.3×10^5	0
6	2.1 W/mL, 5.7 min	101.5	32.9	28.09	4.3×10^5	0
7	1.4 W/mL, 5 min	66.9	150.8	6.07	4.3×10^5	220
8	2.1 W/mL, 4.3 min	78.6	72.6	12.44	4.3×10^5	0

F_{Trp}: Fluorescence of tryptophan; C_0 and C: Initial and final concentration of *E. faecalis*, respectively (CFU/mL).
a) Nonsignificant according to the microbiology counting.

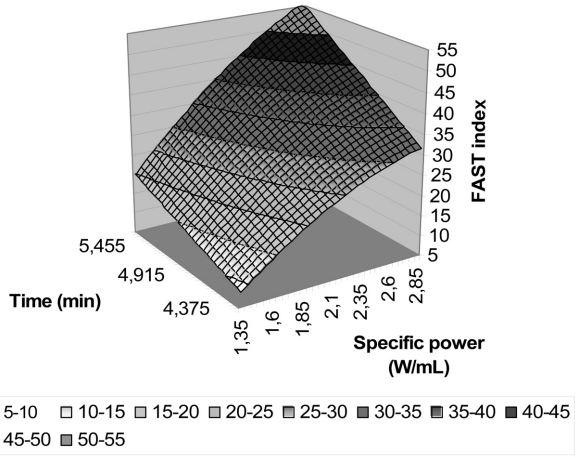

Figure 5. Response surface of the FAST index: Influence of the time of treatment and the specific power.

of MRP) can take up to 24 h and also require sophisticated instrumentation and more complex training of the analyst.

Our experimental design was also set up to find the optimal parameters (*i. e.*, the best specific power and time of treatment) for the microwave pasteurization of milk. The total destruction of *E. faecalis* was the selected criterion to ensure a perfect pasteurization of milk. As shown in Table 1, the microbial count indicated an absence of *E. faecalis* in most of the samples except for samples 1 and 7 which received the weakest microwave treatments. Therefore, we can conclude that in our conditions the best pasteurization treatment which ensured a total elimination of the selected bacteria with the lowest damage of the milk nutritional quality was obtained by a specific power of 1.6 W/mL and a time of treatment of 5.5 min. However, the FAST index and the tryptophan fluorescence measured from the milk sample treated in these conditions (sample 2) indicate that the occurrences of heat damage in proteins were more important in our experimental pasteurization compared to that observed in pasteurized or microfiltrated commercial milks. In other words we assume that we have not yet preserved the same nutritional quality as existing pasteurized milk. Three main reasons have been identified to explain why our FAST index result was higher than those of commercial milks. First of all *E. faecalis* was selected to set up microwave pasteurization in this study because of its non-pathogenic characteristics. However, this microorganism is slightly more heat-resistant than *M. tuberculosis* which is the pathogenic reference for pasteurization [16]. The decimal reduction times at 60°C of *M. tuberculosis* and 70°C of *E. faecalis* are 166 and 177 s, respectively [17]. The difference observed between sample 2 and commercial milks could be explained by the severe pasteurization treatment needed to destroy *E. faecalis.* Moreover, the laboratory-scale instrument used in this study and the long duration of the cooling phase are two other explanations for the inconclusive results of this first experimental design.

4 Concluding remarks

The FAST method appears to be a well adapted tool for the determination of the correct settings that will yield the best nutritional quality of milk when treated by alternative pasteurization technologies such as microwave. The FAST method provides the advantages of being precise, time-saving, and cost-effective. In a coming work we will extend the scope of the study and will examine the effect of microwave treatment with higher microwave specific power, shorter treatment and cooling times.

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5 References

[1] Council directive 92/46/EEC, 2004, Annex C, p. 31.

[2] Grant, I. R., Hitchings, E. I., McCartney, A., Ferguson, F., *et al*., *Appl. Environ. Microbiol.* 2002, *68*, 602–607.

[3] Finot, P. A., *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, in: Finot, P. A., Aeschbacher, H., Hurrell, R. F., Liardon, R. (Eds.), Birkhauser Verlag, Basel 1990, pp. 259–271.

[4] Calvo, M. M., De la Hoz, L., *Int. Dairy J.* 1992, *2*, 69–81.

[5] Rizzi, G. P., *Food Revues Int.* 1997, *13*, 1–28.

[6] Van Boekel, M. A. J. S., *Food Chem.* 1998, *62*, 403–414.

[7] Villamiel, M., Lopez-Fandino, R., Corzo, N., Martinez-Castro, I., *et al*., *Z. Lebensm. Unters. Forsch.* 1996, *202*, 15–18.

[8] Hayes, M. G., Fox, P. F., Kelly, A. L., *J. Dairy Res.* 2005, *72*, 25–33.

[9] Bendicho, S., Barbosa-Cánovas, G. V., Martín, O., *Trends Food Sci. Technol.* 2002, *13*, 195–204.

[10] Kosikowski, F. V., Mistry, V. V., in: Kosikowski, F. V., Westport, L. L. C. (Ed.), *Cheese and Fermented Milk Foods*, FV Kosikowski Llc, Westpoint, Conn., USA 1997, Vol. 1.

[11] Rodriguez, J., Requena, T., Fontecha, J., Goudedranche, H., *J. Agric. Food. Chem.* 1999, *47*, 558–565.

[12] Lombardi, P., Avallone, L., d'Angelo, A., Mor, T., *et al.*, *J. Food Prot.* 2000, *63*, 970–973.

[13] Pellegrino, L., De Noni, I., Resmini, P., *Int. Dairy J.* 1995, *5*, 647–659.

[14] Law, A. J., Leaver, J., *J. Agric. Food Chem.* 2000, *48*, 672–679.

[15] Birlouez-Aragon, I., Sabat, P., Gouti, N., *Int. Dairy J.* 2002, *12*, 59–67.

[16] Veisseyre, R., *Technologie du lait: Constitution, Récolte, Traitement et Transformation du lait*, La maison rustique, Paris 1975, pp. 213–228.

[17] Bazinet, L., Pouliot, Y., Castaigne, F., *Opérations Unitaires*, in: Vignola, C. (Ed.), *Science et Technologie du lait: Transformation du lait*, Presses internationales polytechnique, Canada 2002, pp. 153–275.

