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Original article

Influence of heating conditions in continuous-flow microwave or tubular heat exchange systems on the vitamin B_1 and B_2 content of milk

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Abstract — The effect of continuous-flow microwave heating of milk on the stability of vitamins B_1 and B_2 was determined by ion-pair reverse-phase high-performance liquid chromatography. Results were compared with those obtained using a conventional process having the same heating, holding and cooling phases. When milk was heated in a continuous microwave heating system, at 90 °C without a holding phase, no vitamin B_1 and vitamin B_2 losses were observed. However, when the holding time was raised to 30 s or 60 s, while the content of vitamin B_2 was not modified, the content of vitamin B_1 was lowered (3% and 5%, respectively). Analogous results were obtained when the milk was submitted to a similar heating process using a conventional system. These results indicate that continuous-flow microwave heating of milk at high temperature does not offer any advantage with respect to the vitamin B_1 and B_2 retention compared with a conventional heating process having the same heating, holding and cooling times.

vitamin B_1 / vitamin B_2 / milk / microwave heating

Résumé — Étude de l'effet des différentes conditions de chauffage dans un micro-ondes à flux continu et dans des systèmes tubulaires d'échange de chaleur sur la teneur en vitamines B_1 et B_2 du lait. L'effet du chauffage dans un micro-ondes à flux continu sur la stabilité des vitamines B_1 et B_2 du lait a été déterminé par HPLC en phase reverse. Il a été comparé aux résultats obtenus par la méthode conventionnelle avec les mêmes phases de chauffage, maintien à température et refroidissement. Le chauffage du lait dans un micro-ondes à flux continu à une température de 90 °C et sans maintien à température ne conduit pas à des pertes en vitamines B_1 et B_2. Par contre, si le temps de maintien à température est de 30 ou 60 s, la teneur en vitamine B_2 est maintenue tandis que celle en vitamine B_1 est diminuée (3 % et 5 % respectivement). Des résultats similaires ont été obtenus en utilisant la méthode conventionnelle. Cette étude met en évidence que le chauffage du lait dans un micro-ondes à flux continu n'offre aucun avantage sur la conservation des vitamines B_1 et B_2

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en comparaison à la méthode conventionnelle utilisant les mêmes phases de chauffage, maintien à température et refroidissement.

vitamine B_1 / vitamine B_2 / lait / chauffage par micro-ondes

1. INTRODUCTION

The technique of heating using microwaves offers a number of advantages over conventional methods of processing foods. This is mainly due to the rapid temperature rise and to the ability of microwaves to penetrate the product and heat the bulk of the food [4]. However, although microwaves could be used in any unit process involving the application of heat, in practice other forms of heating may be more suited for technical or economic reasons. Advantages and disadvantages must be carefully considered to ensure a successful application of microwaves [6].

The heating process using microwaves has been described as offering great potential benefits to the dairy industry. Processes where microwaves can be applied include tempering, pasteurisation, sterilisation, cooking and drying [22, 26]. The introduction of alternative methods for heating milk has underlined the importance of evaluating the effects produced as a result of these new processes, in order to establish the conditions that provide the desired degree of safety with a minimum loss in product quality. In this sense, microwave heating of milk has been studied primarily to determine inactivation of milkborne pathogens [1–3, 5] and to evaluate its suitability for use in pasteurisation [7–10, 12, 24, 25]. Thus, in a recent study, the use of a continuous microwave system, including a holding phase to maintain the time and temperature conditions achieved in the microwave oven, proved to be an effective system for pasteurising milk [10].

It is well known that milk is a very rich source of vitamins and it contributes significantly to the dairy intake of most of them [13, 14, 20]. However, due to the fact that vitamins are very sensitive nutrients, the most serious threat to the nutritional value of milk by processing is the destruction of these nutrients [15]. Like every heat treatment, microwave energy can influence the vitamin content of milk. A detailed review about the effect of heating milk in domestic microwave ovens on several vitamins can be found in Sieber et al. [17]. More scarce is the information found in the literature about the effect of continuous-flow microwave processing of milk on its vitamin content. In a previous paper [18] continuous-flow microwave heating of milk at 85 °C was compared favourably with a conventional heating process using a laboratory scale plate heat exchanger at 80 °C, because it produced lower vitamin B_1 losses (0 and 4% loss, respectively). Taking into account that the conventional system had higher time requirements to achieve the prescribed heating temperature, these results were attributed to the shorter residence time for the milk in the microwave system as well as the lack of hot surfaces contacting the milk. However, as has been proved in the case of other milk nutrients, there is no evidence of less destruction of vitamin B_1 taking place during the come-up time when milk is heated at higher temperatures whit a microwave system as compared with a conventional unit having the same residence time.

In the case of other milk vitamins, i.e. vitamin B_2, the stability of this vitamin in buffered solutions heated with microwaves and conventional systems has been demonstrated previously [21]. In milk, vitamin B_2 losses after microwave heating using a batch

process have no been observed [11, 17] however, as in the case of vitamin B_1, there are no studies about the effect of continuous-flow microwave heating of milk as compared with a conventional unit having the same residence time.

The objective of the present work was to study the effect of continuous-flow microwave heating of milk at high temperature on vitamin B_1 and vitamin B_2, and to compare it with a conventional heating process under similar conditions and with the batch microwave heating [11, 23].

2. MATERIALS AND METHODS

2.1. Milk samples

Raw cow's whole milk was obtained from a local farm. Milk was kept refrigerated at 5 °C until it was processed.

2.2. Microwave heating system

A continuous-flow microwave heating system was set up using a 2450 MHz MDS-2000 oven (CEM Corporation, Buckingham, UK), set to operate at 100% power. A coil Teflon tubing with an internal diameter of 0.5 cm and 200 cm in length (39.26 cm^3 of volume) was introduced into the oven cavity through two holes of 0.7 cm diameter drilled in the lower left side of the oven. Inlet and outlet temperatures were continuously monitored using Digiten D 2000 digital thermometers positioned just outside the cavity. The milk, initially at 20 °C, was pumped through the system using a Variable Speed Tubing Pump (Millipore, Bedford, Mass., USA), at a flow rate of 95.2 $mL \cdot min^{-1}$, which provided an outlet temperature of 90 °C (Experiment A, Tab. I). The milk leaving the oven was either cooled immediately or pumped through insulated Teflon tubing of variable lengths (42.8 cm and 84.17 cm) with an internal diameter of

Table I. Heating conditions used in the continuous-flow microwave and conventional heating systems.

Tableau I. Conditions de chauffage appliquées dans le micro-ondes à flux continu et dans le système d'échange de chaleur conventionnel.

Experiment	Flow ($mL \cdot min^{-1}$)	Ti[a] (°C)	tr[b] (s)	th[c] (s)	To[d] (°C)
Microwave heating					
A	95.2	20	24.6	0	90
B	95.2	20	24.6	30	90
C	95.2	20	24.6	60	90
Conventional heating					
D	95.2	20	24.6	0	90
E	95.2	20	24.6	30	90
F	95.2	20	24.6	60	90

[a] Ti: inlet milk temperature; [b] tr: residence time; [c] th: holding time; [d] To: outlet milk temperature. Mean values of the three experiments for each heating system (coefficient of variation ≤ 1.0%).

[a] Ti : température du lait à l'entrée ; [b] tr: temps de séjour ; [c] th : temps de maintien à température ; [d] To : température du lait à la sortie. Valeurs moyennes de 3 expériences pour chaque système de chauffage (coefficient de variation ≤ 1,0 %).

0.8 cm (47.6 cm^3 and 84.17 cm^3 volumes), in order to provide either 30 s or 60 s of holding time at the flow rate used (Experiments B and C, Tab. I). For rapid cooling of milk a 0.4 cm × 200 cm coil of Teflon tubing immersed in an ice/water bath was used.

2.3. Conventional heating system

The conventional heating system (tubular heat exchanger) was set up exactly in the same manner except for the fact that the heating section was replaced by a coil of stainless steel tubing, with an internal diameter of 0.45 cm and length of 246 cm (39.26 cm^3 of volume), and a wall thickness of 0.93 mm, immersed in a temperature-controlled water bath. The temperature of the water bath was adjusted to provide the same outlet temperatures as the microwave oven under the same flow rate conditions, thus ensuring the same heating rate (Experiments D–F, Tab. I).

All of the experiments (A–F) were repeated 3 times with the same raw milk sample.

2.4. Analytical determination

2.4.1. Extraction procedure

An acid and enzymatic extraction procedure for vitamins B_1 and B_2 was carried out according to Sierra et al. [19]. Milk (10 mL) was hydrolysed with 0.3 $mol \cdot L^{-1}$ HCl (30 mL) in an autoclave at 121 °C for 20 min. After cooling to ambient temperature and pH adjustment to 5–5.5 with 4 $mol \cdot L^{-1}$ sodium acetate, 5 mL of 20% aqueous solution Taka-Diastase from *Aspergillus oryzae* (Serva Feinbiochemica GmbH & Co., Heidelberg, Germany) was added and the sample was incubated at 45 °C for 3 h. The sample solution was filtered through No. 40 Whatman filter paper and filled up with distilled water to 100 mL. An aliquot was filtered through a 0.22 mm pore size nylon filter membrane and analyzed by HPLC.

2.4.2. Preparation of standards

Individual standard stock solutions of vitamin B_1 (thiamin) (Sigma Chemical Co., St. Louis, USA) and vitamin B_2 (riboflavin) (Merck, Darmstadt, Germany) were prepared by dissolving appropriate amounts of each one in HCl 0.01 $mol \cdot L^{-1}$. These stock solutions were stable if stored at –20 °C without light for at least 3 months. Four stock solutions of thiamin and riboflavin were made by suitable dilutions in HCl 0.01 $mol \cdot L^{-1}$. The overall concentration ranges were 6.20–49.00 $ng \cdot mL^{-1}$ for thiamin and 40–320 $ng \cdot mL^{-1}$ for riboflavin.

2.4.3. Thiamin HPLC analysis

Analysis of thiamin by HPLC was carried out according to Sierra et al. [19].

Apparatus: A Waters Associates Chromatograph (Waters Associates, Milford, CT), equipped with both a model 510 and M 45 HPLC pumps, a Rheodine sample injector, a mBondapak C_{18} column (300 × 3.9 mm i.d. and 10 mm particle size), a C_{18}/Porasil B Bondapak guard-column (20 × 3.9 mm i.d.) and a Waters 470 scanning fluorescence detector set at 360 nm (excitation) and 435 nm (emission) wave length were employed. The detector signal was recorded on a Maxima 820 Chromatography Workstation (Waters Associates).

Chromatographic conditions: The mobile phase methanol/water/acetic acid (31/68.5/0.5), containing 5 $mmol \cdot L^{-1}$ sodium hexanesulphonate (Sigma) was pumped at a flow rate 1.5 $mL \cdot min^{-1}$. The column temperature was 35 °C and the injection volume were 50 mL.

Post column derivatization: An additional model M 45 pump was employed to pump derivatization reagent (0.001 $mol \cdot L^{-1}$ potassium hexacyanoferrate (III) in 0.25 $mol \cdot L^{-1}$ NaOH) into the eluent stream leaving the column through a T-junction piece, at flow rate 0.7 $mL \cdot min^{-1}$. A stainless-steel

reaction coil (3.0 m × 0.5 mm i.d.) was used. The derivatization reagent was prepared every day from 0.03 mol·L^{-1} potassium hexacyanoferrate (III) aqueous stock solution, 1 h before starting analysis, and was used within the next 6 h. Peak identification was based on the comparison of retention time of standards, as well as by spiking peaks. Calibration curves were obtained by plotting peak height versus concentration with standard solutions subjected to the extraction procedures described above. The correlation coefficients obtained were superior to 0.990.

2.4.4. Riboflavin HPLC analysis

Analysis of riboflavin by HPLC was carried out according to Sierra et al. [19].

Apparatus: A Waters Associates chromatograph, equipped with a model 510 HPLC pump, a Rheodine sample injector, an ODS2 Spherisorb column (300 × 3.9 mm i.d. and 10 mm particle size), a C_{18}/Porasil B Bondapak guard-column (23 × 3.9 mm i.d.) and a Waters 470 scanning fluorescence detector set at 445 nm (excitation) and 520 nm (emission) wave length were employed. The detector signal was recorded on a Maxima 820 Chromatography Workstation (Waters associates).

Chromatographic conditions: A mobile phase methanol/water/acetic acid (31/68.5/0.5), containing 5 mmol·L^{-1} sodiumhexanesulphonate (Sigma) at a flow rate 1 mL·min^{-1} was used. The column temperature was 35 °C and the injection volume was 50 mL. Peak identification was based on the comparison of retention times, as well as by spiking with standards.

The peak heights of riboflavin in sample extracts were measured and compared with standards. Calibration curves were obtained by plotting peak height versus concentration with standard solutions subjected to the extraction procedures described above. The correlation coefficients obtained were always superior to 0.990.

2.5. Statistical analysis

Data obtained from the chemical analysis of the samples were subjected to multifactor analysis of variance (ANOVA) by a statistical program (Statgraphics Graphics System 5.0 Computer Software).

3. RESULTS AND DISCUSSION

Table II collects the content of vitamins B_1 and B_2 in raw milk and milk heated using the continuous-flow microwave system and the tubular heat exchanger (Experiments A–D).

The vitamin B_1 content of the control milk was 0.28 mg·L^{-1}, which is in agreement with previously published data for raw milk (0.3 to 0.6 mg·kg^{-1}) [13, 20]. When control milk was heated with the continuous-flow microwave system at 90 °C without a holding phase (Experiment A), the vitamin B_1 content was not significantly modified ($P \leq 0.05$). However, the same heat treatment but applying holding times of 30 s and 60 s (Experiments B and C) led to significant vitamin B_1 losses, which increased with increased holding time (i.e. 97% and 95% retention, respectively).

When the milk was subjected to analogous treatment using the conventional tubular heat exchanger (90 °C for 0 s, 30 s and 60 s), the vitamin B_1 retention values were similar to those reported above, so there were no significant differences ($P \leq 0.05$) between the vitamin B_1 content of milk heated either with the continuous-flow microwave unit or with the tubular heat exchanger (Experiments D, E and F compared to experiments A, B and C, respectively).

There is not much data in the literature on the extent of vitamin B_1 losses during continuous-flow microwave processing of milk. In a previous paper [18] we have reported bigger losses of vitamin B_1 when milk was heated using a conventional plate

Table II. Vitamins B_1 and B_2 content in milk heated under similar conditions in a continuous-flow microwave and a conventional heating system.

Tableau II. Teneur en vitamines B_1 et B_2 du lait chauffé dans le micro-ondes à flux continu et dans le système conventionnel.

Milk sample	Holding time (s)	Vitamin B_1* ($mg \cdot L^{-1}$)	Vitamin B_2* ($mg \cdot L^{-1}$)
Raw	....	0.279 ± 0.002^a	1.679 ± 0.009^a
Microwave heating			
A	0	0.277 ± 0.001^a	1.678 ± 0.011^a
B	30	0.271 ± 0.001^b	1.672 ± 0.021^a
C	60	0.266 ± 0.002^c	1.655 ± 0.008^a
Conventional heating			
D	0	0.276 ± 0.003^a	1.682 ± 0.061^a
E	30	0.270 ± 0.002^b	1.675 ± 0.017^a
F	60	0.265 ± 0.001^c	1.688 ± 0.009^a

* Values are the mean of nine determinations ± standard deviation. The same superscripts in the same column for each vitamin indicate no significant differences ($P \leq 0.05$).

* Les valeurs proviennent de la moyenne de 9 déterminations ± écart-type. Dans chaque colonne, les mêmes lettres en exposant indiquent qu'il n'y a pas de différence significative ($p \leq 0,05$) entre les traitements.

heat exchanger at 80 °C compared to a continuous-flow microwave unit at 85 °C (0 and 4% losses, respectively). These results were attributed to the shorter residence time needed to achieve the prescribed temperature when milk was heated in the microwave system (come-up time equal to 16.5 s), in comparison with the 70.99 s needed with the plate heat exchanger. Besides this, due to the fact that heating of milk in the conventional system takes place through heat-transfer surfaces, the bigger vitamin B_1 losses were also attributed to milk overheating.

Results obtained in the present work indicate no significant differences in the vitamin B_1 content of milk heated either with the tubular heat exchanger or with the continuous-flow microwave oven (Experiments A and D). Because the come-up time was similar in both cases, these results indicate that the hot surfaces of the tubular exchanger in contact with the milk do not produce vitamin B_1 losses. The higher time needed to achieve the prescribed temperature appears to be the main cause of the vitamin losses observed when milk was heated using a conventional plate heat exchanger [18].

The vitamin B_2 content of control milk was 1.68 $mg \cdot L^{-1}$ (Tab. II), which is within the range of 1.4 to 2.3 $mg \cdot kg^{-1}$ reported in the literature for raw milk [13, 20]. Microwave or conventional heating to 90 °C for 0 s, 30 s and 60 s did not significantly modify ($P \leq 0.05$) the content of this vitamin. This is confirmation of the previously reported thermal stability of this vitamin [16, 17] and indicates that continuous-flow microwave heating of milk does not cause any additional destructive effect. In this way, van Zante and Johnson [21] found no significant differences between buffered solutions of vitamin B_2 heated with microwaves or conventional systems.

The literature on the effect of microwave heat treatment of milk on vitamins is inconsistent and, generally, values cannot be

compared. This is mainly due to the fact that published works report different conditions of heat treatment, type and volume of milk, power of the oven and temperature and time of exposure. Vidal-Valverde and Redondo [23] reported a 55% vitamin B_1 loss when 150 mL of commercial UHT whole milk was heated in a domestic microwave oven at 80 °C. However, in a similar study carried out by Sieber et al. [16], these authors did not found vitamin B_1 losses in the upper and lower parts of raw milk microwave-heated to 78 °C (batch method) and in stirred or unstirred milk. In another study, the same authors observed no vitamin B_1 losses after batch microwave heating of pasteurised milk at 83 °C with a holding time of 4 min [17].

As in the case of vitamin B_1, no studies have been carried out on the effect of continuous-flow microwave heating of raw milk over its vitamin B_2 content. Medrano et al. [11] indicated that batch microwave heating of UHT whole milk at 80 °C does not produce significant vitamin B_2 losses. Vitamin B_2 losses were not found after heating commercial pasteurised whole milk at a temperature of 83 °C with a holding time of 4 min [17].

To summarise, results of the present work indicate that continuous-flow microwave heating of milk does not show a clear advantage on the retention of vitamins B_1 and B_2 compared with a conventional heating system having the same heating, holding and cooling times. However, considering the effect on other nutrients present in milk [26], continuous-flow microwave treatments of milk could still be advantageous.

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