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Riboflavin, α-tocopherol and retinol retention in milk after microwave heating

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Summary — The effect of microwave heating during 2–4 min on the riboflavin, α-tocopherol and retinol content of milk, with three different fat levels, was studied by HPLC. The riboflavin content was not significantly modified by this treatment. Heating during 2 min did not modify the α-tocopherol content of whole milk, but in the case of low fat milk, the α-tocopherol content significantly decreased. The effect on the retinol content was less pronounced but still significant ($P < 0.05$). Further heating (up to 4 min) did not modify the vitamin content in any case.

milk / microwave heating / riboflavin / α-tocopherol / retinol

Résumé — Rétention des vitamines riboflavine, α-tocophérol et rétinol dans le lait après chauffage par micro-ondes. *L'effet du chauffage par micro-ondes, pendant 2 ou 4 min, sur le contenu en riboflavine, α-tocophérol et rétinol du lait entier, demi-écrémé ou écrémé, a été étudié par HPLC. Le contenu en riboflavine n'est pas modifié significativement par ce traitement. Le chauffage pendant 2 min ne modifie pas la quantité d'α-tocophérol du lait entier, mais son contenu diminue significativement dans le lait demi-écrémé. L'effet sur le contenu en rétinol est moins important, mais significatif (P < 0,05). Un chauffage plus prononcé (jusqu'à 4 min) ne modifie plus la quantité des vitamines.*

lait / chauffage par micro-ondes / riboflavine / α-tocophérol / rétinol

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INTRODUCTION

Microwave processing can offer several distinct advantages when compared to conventional heating methods.

These advantages include speed of operation, energy savings, precise process control, etc. Speed of operation is the primary advantage. Since microwaves penetrate within a food and not just at the surface, heating occurs more rapidly. This accelerated heating provides for a higher quality product in terms of taste, texture and nutritional content (Giese, 1992). Microwave sterilization can deliver products that taste good because microwaves are able to heat the product 3–5 times faster than conventional sterilization systems (Halfinger, 1992).

The domestic use of microwave ovens to heat different foods has been widely extended during the last few years, despite its numerous problems of uneven cooking, lack of browning and crisping, etc. It is rarely a cooking tool, but rather a rapid convenient reheater which is used so frequently during the day that consumers are not even aware of it (Schiffmann, 1992). This is particularly the case of milk feeding bottles, normally heated by microwave oven because of the speed and convenience.

It is assumed than the nutritive value of food is scarcely affected after microwave heating. Few data exist on the effects of microwave heating on the vitamin content of milk (Cross and Fung, 1982; Vidal-Valverde and Redondo, 1993). It is known that tocopherols are sensitive to oxidation, heat and light. Heat and ionizing radiations are also known to calalyze autoxidation processes (Cross and Fung, 1982) which have also been presumed to be taking place during processing and storage of retinol and tocopherol-containing foods (Packer *et al,* 1981; Vidal-Valverde *et al,* 1992, 1993).

The purpose of this work was to study the riboflavin, α-tocopherol and retinol content of cow milk containing three levels of fat as well as the effect of microwave heating, using domestic conditions, on the retention of riboflavin, α-tocopherol and retinol.

MATERIALS AND METHODS

Three types of commercial cow UHT milk containing different levels of fat have been studied: whole milk (3.3%); low fat milk (1.45%); and skim milk (0.2%). Four lots, consisting of three 150-ml milk samples of whole milk, low fat milk and skim milk respectively, were exposed to 700 W (2450 MHz) microwave treatment (EM-780 Sanyo Electric Co, Osaka, Japan), during 2 min in the dark. Another four lots, prepared in the same manner, were submitted to the same heat treatment, for 4 min. The samples were quickly cooled in an ice bath and finally frozen for storage. Control samples not subjected to microwave heating were also frozen (–20°C) in glass bottles protected from the light. Storage time never exceeded 60 days before analysis (Vidal-Valverde *et al,* 1992, 1993).

Table I summarizes the conditions of milk treatment with the microwave oven. The times of heating and the increase of milk temperature during the process are given. Domestic conditions were used in this study.

Extraction of α-tocopherol and retinol

α-Tocopherol and retinol of control and heated samples of milk were determined by HPLC (Vidal-Valverde *et al,* 1992, 1993). Milk (10 ml), protected from light, was mixed with ascorbic acid solution (50 ml, 5% w/v) and potassium hydroxide solution (10 ml, 50% w/v) in an 500-ml Erlenmeyer flask. The mixture was heated for 20 min in a water-bath (80–90°C), with stirring under reflux conditions in a nitrogen atmosphere. This solution was repeatedly extracted with diethyl ether in a separatory funnel (150 ml total volume). Butylated hydroxytoluene (5 mg) was added to the ether extract. The ether was removed in a rotary evaporator, under nitrogen atmosphere, in a water-bath at temperature lower than 40°C. Ethanol (10 ml) was added to the residue and removed again under vacuum. Finally, the residue was dissolved in methanol (5 ml), filtered through an 0.45-μm membrane (HAWP, Millipore) and quantified by HPLC.

Table I. Heating time, initial and final temperature of milk achieved with a microwave oven.
Temps de chauffage, températures initiales et finales du lait obtenues avec un four à micro-ondes.

Sample	*Time min*	*Initial temperature (°C)*	*Final temperature (°C)*	*Temperature increase (°C)*
Whole milk				
Treatment A	2	19.6 ± 0.54	56.2 ± 1.67	33.0 ± 1.58
Treatment B	4	20.2 ± 0.83	80.2 ± 3.42	60.0 ± 3.60
Low fat milk				
Treatment A	2	19.6 ± 0.54	53.2 ± 0.84	33.6 ± 1.14
Treatment B	4	20.2 ± 0.84	79.6 ± 2.07	59.4 ± 1.52
Skim milk				
Treatment A	2	19.6 ± 0.55	53.6 ± 1.14	34.0 ± 1.58
Treatment B	4	20.1 ± 1.00	76.6 ± 1.82	56.8 ± 2.16

Means values and standard deviation (n = 4); moyenne des 4 déterminations ± écart type.

The extraction and quantification method here described (which includes a saponification step), allows only for the analysis of total retinol content. The same could be stated about the tocopherol esters. However, in the analysis of unfortified milk, this is not important since tocopherol esters are not present in natural foods.

Extraction of riboflavin

Milk (10 ml), protected from light, and 0.3 N HCl (10 ml) was well mixed and autoclaved for 20 min at 121°C. After cooling to ambient temperature and pH adjustment to 4.5 the samples were filtered through No 40 ashless Whatman filter paper and filled up with distilled water to 100 ml. An aliquot was filtered through an 0.45-µm membrane (HAWP, Millipore) and analysed by HPLC.

HPLC determination

HPLC analysis of α-tocopherol and retinol were carried out using a chromatograph model KNK-500 (Konik Instruments SA) equipped with a model KNK-029375 gradient controller and a model KNK-029757 UV/VIS detector. Data were processed on a model SP-4290 computer integrator (Spectra-Physics, CA, USA).

Stock solutions of DL-α-tocopherol containing 31.4 mg of DL-α-tocopherol in 250 ml of methanol and 10.38 mg of retinol in 100 ml of methanol were prepared. Different standard solutions ranging between 0.3 and 5.02 µg/ml of the α-tocopherol and between 0.2 and 4.15 µg/ml of retinol were prepared from the above stock solutions. The standard solutions were protected from light.

HPLC determination of riboflavin was carried out using a Model M-510 pump (Waters Associates), a Rheodyne M-7125 injector (Cotati, CA, USA) and a Model 470 fluorescence detector (Waters Associates). Results were processed on PC NEC Power Mate 1 with a Maxima database (Millipore Corporation, Waters Chromatography Division, Wilford, CT, USA). Riboflavin standard solutions (0.1, 0.2 and 0.4 µg/ml) were prepared every week from a stock riboflavin solution containing riboflavin (10 mg) in 0.01 N HCl (500 ml). The standard solutions were protected from light.

The chromatographic conditions are summarized in table II. Peak identification was based on the comparison of retention times as well as by

Table II. Chromatographic conditions.
Conditions chromatographiques.

Vitamin	*Guard column*	*Type column*	*Temperature column*	*Mobil phase*	*Flow rate*	*Detector wavelenght*
α-Tocopherol	–	Spherisorb ODS2 25 x 0.46 cm (5 µm)	30°C	Methanol/water (98:2)	1 ml/min	295 nm (UV)
Retinol	–	Spherisorb ODS2 25 x 0.46 cm (5 µm)	30°C	Methanol/water (98:2)	1 ml/min	325 nm (UV)
Riboflavin	C18 Porasil B (Waters Assoc) 3.4 x 22.7 mm	Spherisorb ODS2 30 x 0.39 cm (10 µm)	Ambient	Methanol/water/acetic acid (31:68.5:0.5) 5 mmol/l sodium hexanesulphonate	1.2 ml/min	450/530 nm (exc/em)

spiking with the standards. The peak area of α-tocopherol and retinol, and the height of riboflavin in sample extracts were measured and compared with standards. Calibration curves were obtained by plotting peak areas or height *versus* concentration with standard solutions subjected to the extraction procedures described above. The correlation coefficients obtained were > 0.990.

Statistical methods

Multifactorial analysis of variance was applied to the data using Statgraphics, Statistical Graphics System 2.1 (Statistical Graphics Corporation, Rockville, MD, USA) with an IBM Personal System/2 Model 20 Computer (International Bussines Machines Corporation, UK).

RESULTS AND DISCUSSION

Three types of cow milk with three levels of fat (3.3%, 1.45% and 0.2%) were studied. The riboflavin, α-tocopherol and retinol content of whole, low fat and skim milk are given in table III. The amount of riboflavin ranges between 1.54–1.72 mg/l. The content of liposoluble vitamins α-tocopherol and retinol was related to the content of fat. Thus α-tocopherol and retinol contents were 1.03 and 0.40 mg/l for whole milk, 0.35 and 0.12 mg/l for low fat milk respectively and were not detected in skim milk.

The effect of microwave heating on riboflavin, α-tocopherol and retinol content of whole, low fat and skim milk are given in table III. The riboflavin content was not significantly modified after microwave heating for 2 or 4 min in any type of milk. The α-tocopherol and retinol content of whole milk were also not modified after microwave heating for 2 or 4 min. In the case of low fat milk, heating during 2 min lowered the α-tocopherol content significantly (85.7% retention). The effect on the retinol content was less pronounced (91.7% retention) but still significant ($P < 0.05$). In both cases, further heating (up to 4 min) did not modify the vitamin content. In the case of skim milk, liposoluble vitamins were not detected before and after microwave heating.

There is some information about the effect of microwave heating on the water soluble vitamin content of vegetables and meat. The effect of this treatment depends on the type of food and the conditions of heating (Cross and Fung, 1982; Baldwin, 1983; Gerster, 1989; Yoshida and Kajimoto, 1989). However, bibliographic data about the effect of microwave heating on the vitamin content of milk are very scarce. Vidal-Valverde and Redondo (1993) found that microwave heating of milk partly destroys its thiamin and this effect is directly proportional to temperature and time and inversely proportional to the level of fat. In this study we observed that after microwave heating the retention of riboflavin ranged between 97–100% and no influence was observed for the level of fat of milk.

Regarding the effect of microwave heating on liposoluble vitamins of foods, only a decrease of 10% of α-tocopherol content after microwave heating during 6 min in vegetable oils was observed. The decrease was less pronounced in the case of oils with high amount of polyunsaturated fatty acids than with saturated fatty acids (Yoshida and Kajimoto, 1989; Yoshida *et al,* 1990). We have found no information about the effect of microwave heating on liposoluble vitamins of milk. In this work, we have shown that while no losses occur with whole milk, significant losses of α-tocopherol take place after heating low fat milk for 2 min. This points out a protective effect of fat on the stability of α-tocopherol during microwave heating. The same observation has been made for some other vitamins. Thus Coulter and Thomas (1968) have pointed out that vitamin A is more stable in whole milk products than in low fat or skimmed milk products, presumably because of natural antioxidants present in milk fat. Lau *et al*

Table III. Effect of microwave oven heating on the α-tocopherol, retinol and riboflavin of cow milk*. *Effet du chauffage par micro-ondes sur l'α-tocophérol, le rétinol et la riboflavine du lait de vache**.

Samples	*α-Tocopherol (mg/l)*	*Retention (%)*	*Retinol (mg/l)*	*Retention (%)*	*Riboflavin (mg/l)*	*Retention (%)*
Whole milk	1.03 ± 0.01[a]	100	0.40 ± 0.01[a]	100	1.72 ± 0.02[a]	100
Treatment A	1.00 ± 0.03[a]	97.1	0.39 ± 0.01[a]	97.5	1.71 ± 0.01[a]	99.4
Treatment B	0.99 ± 0.06[a]	96.1	0.40 ± 0.01[a]	100	1.67 ± 0.03[a]	97.1
Low fat milk	0.35 ± 0.003[a]	100	0.12 ± 0.003[a]	100	1.54 ± 0.03[a]	100
Treatment A	0.30 ± 0.01[b]	85.7	0.11 ± 0.004[b]	91.7	1.53 ± 0.01[a]	99.4
Treatment B	0.30 ± 0.01[b]	85.7	0.11 ± 0.002[b]	91.7	1.55 ± 0.02[a]	100.6
Skim milk	ND		ND		1.61 ± 0.003[a]	100
Treatment A	ND		ND		1.65 ± 0.01[a]	102.5
Treatment B	ND		ND		1.63 ± 0.02[a]	101.2

* Mean of 4 determinations ± SD; ND : not detected. Different superscripts within each vitamin and milk indicate significant differences ($P < 0.05$).
* *Moyenne de 4 déterminations ± écart type. ND : non déterminé. Des lettres différentes en indice entre chaque vitamine et lait indiquent des différences significatives au seuil* $P < 0,05$.

(1986) observed that degradation of vitamin A during storage of fortified UHT milk varied inversely with the fat content in the milk. Fellman *et al* (1991) showed that vitamin A was more stable in low fat milk than in skim milk after exposure to light. Vidal-Valverde and Redondo (1993) also found that the degradation of thiamin after microwave heating of milk is inversely proportional to the level of fat. The fact that extended heating (up to 4 min) did not further modify the α-tocopherol content of low fat milk could perhaps indicate that a certain portion of the native α-tocopherol is lost rapidly on microwave treatment, with the remaining portion being resistant to further destruction. A similar observation has been made for light exposure of vitamin A (de Man, 1981).

On the other hand, the data in table III pointed out that retinol is more stable than α-tocopherol after microwave heating. In previous papers (Vidal-Valverde *et al,* 1992, 1993), we have observed the same effect during storage of milk. It is admitted that α-tocopherol is a natural antioxidant (Kanno *et al,* 1970a,b; Cort, 1974; Chow and Draper, 1974) and in this sense it would be possible to assign α-tocopherol a protecting role over retinol.

The conclusion of this study is that the microwave heating, in domestic conditions, does not affect, or only slightly, the riboflavin, α-tocopherol and retinol content of cow milk, and especially in the case of whole milk. Since this milk is a relevant source of vitamin A and E for a part of the population (babies and children) for whom milk is one of the most important parts of their diet, the fact that microwave treatment basically did not affect their content is important from a nutritive point of view.

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