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Free amino acid concentrations in milk: effects of microwave *versus* conventional heating

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Summary. Microwave effects on free amino acid concentrations in milk *versus* a water bath heating were investigated in view of their importance for infant growth. Concentrations of few amino acids, such as aspartate, serine or lysine, are unchanged whatever the way and the temperature of heating. In contrast, tryptophan concentrations decreased similarly whatever the way of heating ($110 \pm 3\mu$mol/l before heating *vs* $84 \pm 4\mu$mol/l after 30°C microwave heating, $p < 0.05$). On the contrary, concentrations of glutamate and glycine increased more after water bath heating at 90°C (325 ± 4 and $101 \pm 1\mu$mol/l, respectively) than after microwave heating (312 ± 4 and $95 \pm 1\mu$mol/l, respectively, $p < 0.05$) suggesting milk proteolysis. Moreover, the accumulation of ammonia observed at 90°C with the water bath together with increase Glu levels might reflect a degradation of glutamine. An ornithine enrichment, more evident with microwave heating, was shown and could be of interest as it is a polyamine precursor. Also, considering few variations of free amino acid concentrations and the time saved, microwave heating appears to be an appropriate method to heat milk.

Keywords: Free amino acids – Milk – Microwave – Water bath heating

Introduction

Recently, Lubec et al. (1989) were involved in a controversy about the effects of microwave heating on milk protein amino acids. In particular, these authors described a microwave heating induced isomerization of L-proline to D-proline, which might be neurotoxic for neonates. Subsequently, microwave heating was reported not to cause racemization of milk amino acids under conventional temperature and pressure conditions (Marchelli et al., 1992; Petrucelli et al., 1994; Finot, 1996). Nevertheless, the use of microwaves for heating is still frequently debated. As far as we know, no data, either quantitative or qualitative, are available on microwave effects on free amino acids in milk. In view of the potential importance of this issue for infant growth, we investigated the free amino acid concentrations of ultra high temperature (UHT) cow milk before and after microwave heating. The results were compared with those obtained with conventional heating, i.e., water bath.

Materials and methods

Two samples (90 mL) of six UHT half-cream milk in bottles (Toury, France) were placed in open glass baby bottles and heated separately in a microwave oven (Moulinex, FM 3940GSB) and in a water bath. When milk temperatures measured with a thermometer were equal to exactly 30°C and 90°C, aliquots of milk were sampled for amino acid analysis. After deproteinization with sulfosalicylic acid (50 mg/ml) and centrifugation, the free amino acid concentrations were quantified by ion exchange chromatography with ninhydrin detection on an amino acid analyzer (model 6300 Beckman, Palo Alto, USA). In parallel, unheated milk controls (n = 6) were taken and similarly treated.

Statistical analysis: all results are expressed as mean ± SEM. Statistical significance was determined by analysis of variance (ANOVA) followed by the Newman-Keüls test using PCSM Software (Deltasoft, Grenoble, France). Differences were considered statistically significant when the $p \leq 0.05$.

Results and discussion

Concentrations of few amino acids such as aspartate, serine or lysine were unchanged whichever the way and temperature of heating, whereas tryptophan concentrations decreased (Table 1). In contrast, concentrations of other amino acids significantly rose after heating (Table 1). These increases were greater at 90°C than 30°C and with water bath heating. They included amino acids present in large amounts in proteins such as glutamate and

Table 1. Concentrations of free amino acids in cow milk before and after heating

Free amino acids µmol/l	Before heating n = 6	After heating			
		30°C		90°C	
		water bath n = 6	microwave n = 6	water bath n = 6	microwave n = 6
Aspartate	25 ± 1	25 ± 0	25 ± 0	27 ± 0	26 ± 0
Serine	17 ± 1	14 ± 1	15 ± 1	15 ± 1	14 ± 0
Glutamate	285 ± 2	303 ± 3*	302 ± 2*	325 ± 4*	312 ± 4■
Proline	20 ± 1	18 ± 1	19 ± 1	20 ± 1	19 ± 1
Glycine	95 ± 1	94 ± 1	95 ± 1	101 ± 1*	96 ± 1■
Alanine	36 ± 1	37 ± 1	37 ± 1	39 ± 1*	37 ± 0■
Valine	7 ± 1	14 ± 1*	13 ± 1	14 ± 1*	14 ± 0*
Tryptophan	110 ± 3	78 ± 1*	84 ± 4*	85 ± 3*	82 ± 3*
Lysine	35 ± 1	36 ± 1	34 ± 1	37 ± 0	35 ± 1
Histidine	18 ± 0	18 ± 1	18 ± 2	19 ± 0	18 ± 1
Arginine	22 ± 1	23 ± 0	25 ± 2	25 ± 1*	23 ± 1

Mean ± SEM: test ANOVA + Newman-Keüls, $^*p < 0.05$ *vs* milk before heating, ■$p < 0.05$ *vs* milk after water bath heating.

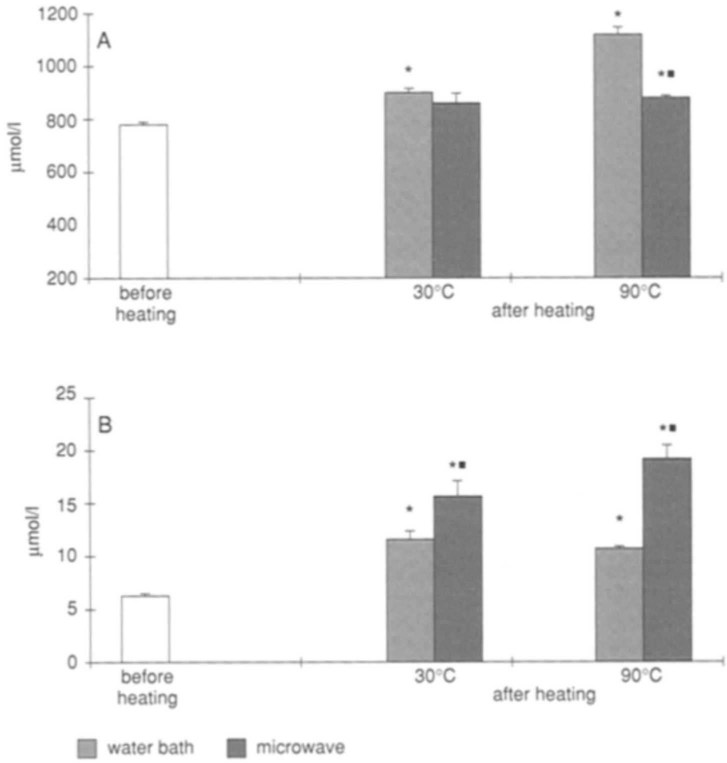

Fig. 1. Free ammonia (**A**) and ornithine (**B**) concentrations in cow milk before and after heating. Mean ± SEM; test ANOVA + Newman-Keüls, *$p < 0.05$ *vs* milk before heating, ■$p < 0.05$ *vs* milk after water bath heating

glycine (Munro, 1964), suggesting proteolysis during milk heating. The fact that proteolysis was greater during water bath heating was probably because a longer heating time was required to reach 90°C: 10 min for the water bath compared with 1.30 min for microwaves.

Milk heating also induced an increase in ammonia concentrations, which was more marked at 90°C with the water bath (Fig. 1A). This accumulation may reflect the degradation of some amino acids, particularly glutamine, which is unstable to heat (Grimble, 1992). This amino acid is broken down to ammonia, known for its neurotoxic properties (Cooper et al., 1985), and glutamate. This could also contribute to the accumulation of glutamate observed during milk heating.

Interestingly, the concentrations of ornithine, an amino acid not present in the protein, were significantly increased at both temperatures and more markedly for the microwave heating (Fig. 1B). This can result from a release of ornithine adsorbed on milk proteins. This ornithine enrichment is potentially relevant because this amino acid is the precursor of polyamines implicated in the immune system and in cell differentiation and multiplication (Pegg, 1986).

Considering the time saved and the results obtained, particularly for ammonia and ornithine, microwave way appears, in our conditions of use, a suitable method to heat cow milk.

Acknowledgements

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References

Cooper AJL, Mora SN, Cruz NF, et al (1985) Cerebral ammonia metabolism in hyperammonemic rats. J Neurochem 44: 1716–1723

Finot PA (1996) Effets du traitement par les micro-ondes sur la qualité nutritionnelle des aliments. Cah Nutr Diét 31:4: 239–246

Grimble G (1992) Glutamine glutamate and pyroglutamate facts and fantaisies. Clin Nutr 11: 66–69

Lubec G, Wolf C, Bartosch B (1989) Amino-acid isomerisation and microwave exposure. Lancet ii (8676): 1392–1393

Marchelli R, Dossena A, Palla G, et al (1992) D-amino-acids in reconstituted infant formula: a comparison between conventional and microwave heating. J Sci Food Agric 59: 217–226

Munro HN (1964) An introduction to biochemical aspects of protein metabolism. In: Munro HN, Allison JB (eds) Mammalian protein metabolism. Academic Press, New York, pp 31–34

Pegg A (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234: 249–262

Petrucelli L, Fisher GH (1994) D-aspartate and D-glutamate in microwaved versus conventionally heated milk. J Am Coll Nutr 13: 209–210

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