Evidence for the Absence of Amino Acid Isomerization in Microwave-Heated Milk and Infant Formulas

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According to a recent paper (Lubec et al., 1989), microwave heating of milk or reconstituted infant formulas could induce the inversion of amino acid residues to a significantly higher extent than conventional heating. In this study, UHT milk and three different infant formulas were heated under two sets of conditions: 600 W for 3 min and 70 W for 20 min. When the proportions of D-amino acids were measured after acid hydrolysis, no significant differences could be found between untreated and treated samples. On the basis of these results, it is concluded that heating of milk or infant formula in a microwave oven under conditions corresponding to those normally applied for heating food does not induce significant inversion of protein-bound amino acids.

INTRODUCTION

Structural and chemical changes occurring in food proteins on processing may produce undesirable nutritional effects. One of the possible changes is amino acid isomerization. The conversion of L-amino acids in food protein into D isomers generates nonutilizable forms of amino acids and creates peptide bonds resistant to proteolytic enzymes (Freimuth et al., 1978; Masters and Friedman, 1980; Friedman et al., 1981; Bunjapamai et al., 1982; Tovar and Schwass, 1983; Jenkins et al., 1984; Friedman and Gumbmann, 1984). A number of studies have been devoted to investigation of the factors that influence the isomerization of free and protein-bound amino acids (Hayase et al., 1973, 1975, 1979; Masters and Friedman, 1979; Friedman and Masters, 1982; Liardon and Hurrell, 1983). The conclusion that may be derived from those studies is that significant isomerization only occurs under excessive conditions of temperature, alkaline pH, and/or treatment time. Temperature and pH prevailing under normal food-processing conditions produce negligible amounts of D-amino acids.

Recently Lubec et al. (1989) reported that microwave heating induced higher racemization rates in food proteins. In particular, D-proline and *cis*-D-hydroxyproline were reported to have been found in significant amounts in microwave-heated infant milk formulas. Allegedly, following absorption these isomers could theoretically be incorporated into peptides and proteins leading to structural, functional, and immunological changes. It was further noted that D-proline had been reported to be neurotoxic when injected in the brain of chicks (Cherkin et al., 1978). On the other hand, there has been no report of D-amino acid residues being incorporated into protein biosynthesis in humans or in any animal species, and the soundness of Lubec's biological and toxicological considerations have been convincingly rebutted (Segal, 1990a,b; Lubec, 1990).

The enhancing effect of microwave heating on amino acid isomerization was apparently supported by the results of Chen et al. (1989), who observed the complete racemization of free amino acids after less than 2 min of microwave irradiation. These results, however, were obtained for amino acids dissolved in acetic acid in the presence of benzaldehyde and heated in sealed vials with the temperature reaching above 200 °C at the end of the irradiation period. Such conditions do not compare well with normal cooking procedures. Therefore, the effect of normal microwave heating conditions on amino acid isomerization remained to be verified. We report here on a study undertaken to this effect using milk and infant formulas heated in open vessels.

MATERIALS AND METHODS

Materials. Products selected for the study were full-cream UHT (Orlait, Switzerland) and three instant formulas produced by two major manufacturers and purchased locally. The protein fraction of these formulas consisted respectively of (A) a mixture of whole milk and demineralized whey, (B) enzymically hydrolyzed whey protein, and (C) a mixture of hydrolyzed animal and soy protein, as well as an unknown proportion of free amino acids. Standard D- and L-amino acids were obtained from Sigma Chemical Co. (St. Louis, MO) and pentafluoropropionic anhydride, acetyl chloride, and Dowex 50WX4 from Fluka (Buchs, Switzerland).

Sample Preparation and Microwave Treatment. The three formulas were reconstituted according to the manufacturer's instructions with bottled water (Evian). UHT milk was used as received. Samples of 150 mL of each product were placed in open glass baby bottles and heated in a Panasonic NN-5557 microwave oven for 3 min at 600 W (final temperature 82–93 °C) or for 20 min at 70 W (final temperature ca. 58–66 °C). At the end of the treatments, the samples were left to cool to room temperature. Aliquots of each sample were kept untreated to references.

Sample Analyses. Samples (1 mL) of treated and untreated products were placed in vacuum reaction ampules (Pierce Chemical Co., Rockford, IL) together with 1 mL of concentrated HCl and hydrolyzed for 24 h at 110 °C as described elsewhere (Liardon and Hurrell, 1983). After the hydrochloric acid was removed on a rotary evaporator, the residue was dissolved in 2 mL of 0.1 N HCl and applied to a small Dowex 500WX4 (H^+) cartridge. The cartridge was washed with 10 mL of distilled water, and the amino acids were displaced with 2 mL of 3 N NH_4OH into conical reaction vials, where they were dried under a stream of nitrogen.

Amino acids recovered from the Dowex cartridges were converted into *N*(*O*,*S*)-perfluoropropionylisopropyl esters in a two-step reaction: (1) esterification in 2-propanol/acetyl chloride followed by (2) acylation with perfluoropropionic anhydride (PFPA). The details of the procedure have already been described (Liardon et al., 1981). After the excess reagent was evaporated, the derivatized amino acids were redissolved in 1 mL of ethyl acetate/hexane (1:20 v/v).

The analyses were performed on a HP 5995A bench-top GC/MS equipped with a 25 m × 0.25 mm (i.d.) Chirasil-L-Val fused silica capillary column (Applied Science Labs, Deerfield, IL). Sample introduction (2 μL) was made in split mode, with a 20:1 split ratio and an injector temperature of 250 °C. Column tem-

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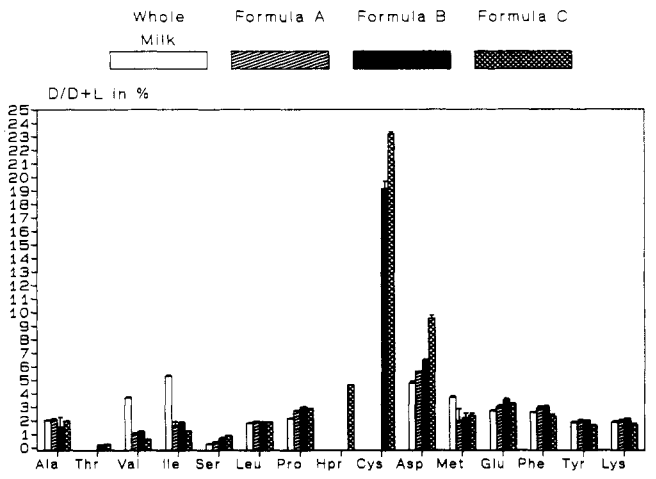

Figure 1. Amino acid isomerization induced during the hydrolysis of the untreated products (segments on top of the bars are standard deviations).

perature was programmed from 80 (hold time 4 min) to 190 °C at 3 °C/min with a final hold time of 8 min. Helium was used as carrier gas with an inlet pressure of 10 psi. Ion source temperature was set at 250 °C, and the ionizing voltage was 70 eV. The mass spectrometer was run in the selected ion monitoring mode (SIM). All analyses were run in duplicate.

The extent of isomerization was calculated as the abundance of each amino acid D isomer relative to the sum of the D and L isomers. For amino acids possessing two asymmetric centers (Ile, Thr, and Hpr) isomerization was calculated by using the following ratios: D-allo-Ile/(D-allo-Ile + L-Ile), D-allo-Thr/(D-allo-Thr + L-Thr), and *cis*-4-OH-D-Pro/(*cis*-OH-4-D-Pro + *trans*-OH-4-L-Pro). In the case of hydroxyproline (Hpr), the four possible stereoisomers (cis-D, cis-L, trans-D, and trans-L) were completely resolved under our chromatographic conditions.

RESULTS AND DISCUSSION

Samples of UHT milk and reconstituted formulas were heated under conditions corresponding to those applied at home for warming up or cooking food (600 W for 3 min), as well as at a lower power (70 W) for a prolonged time (20 min). The latter conditions were applied as an attempt to distinguish an intrinsic microwave effect from the thermal effect. The samples were placed in open glass bottles rather than in sealed vessels to avoid excessive temperature rise and pressure buildup. Amino acid isomerization was measured after hydrolysis of the samples. This process is known also to induce some isomerization to varying extent depending on the nature of the amino acid (Liardon et al., 1981). Therefore, untreated samples were analyzed in the same way, and the contribution of microwave treatment to amino acid isomerization was evaluated by comparing treated and untreated samples.

The extent of hydrolysis-induced racemization in the four untreated products is shown in Figure 1. In general, the differences between the various products are hardly significant. The exception is the higher isomerization rate of Val, Ile in UHT milk, and Asp in formula C. Evidence for such an influence of the nature of the protein on the hydrolysis-induced isomerization of particular amino acid residues has been presented in earlier studies (Liardon and Jost, 1981; Liardon and Ledermann, 1984). The absence of racemization value for hydroxyproline in UHT milk and in formulas A and B is due to the very low concentration of this amino acid in these products which did not permit the detection of the D isomer. By contrast, the two isomers of hydroxyproline were readily detectable in formula C, which is suspected to contain hydrolyzed

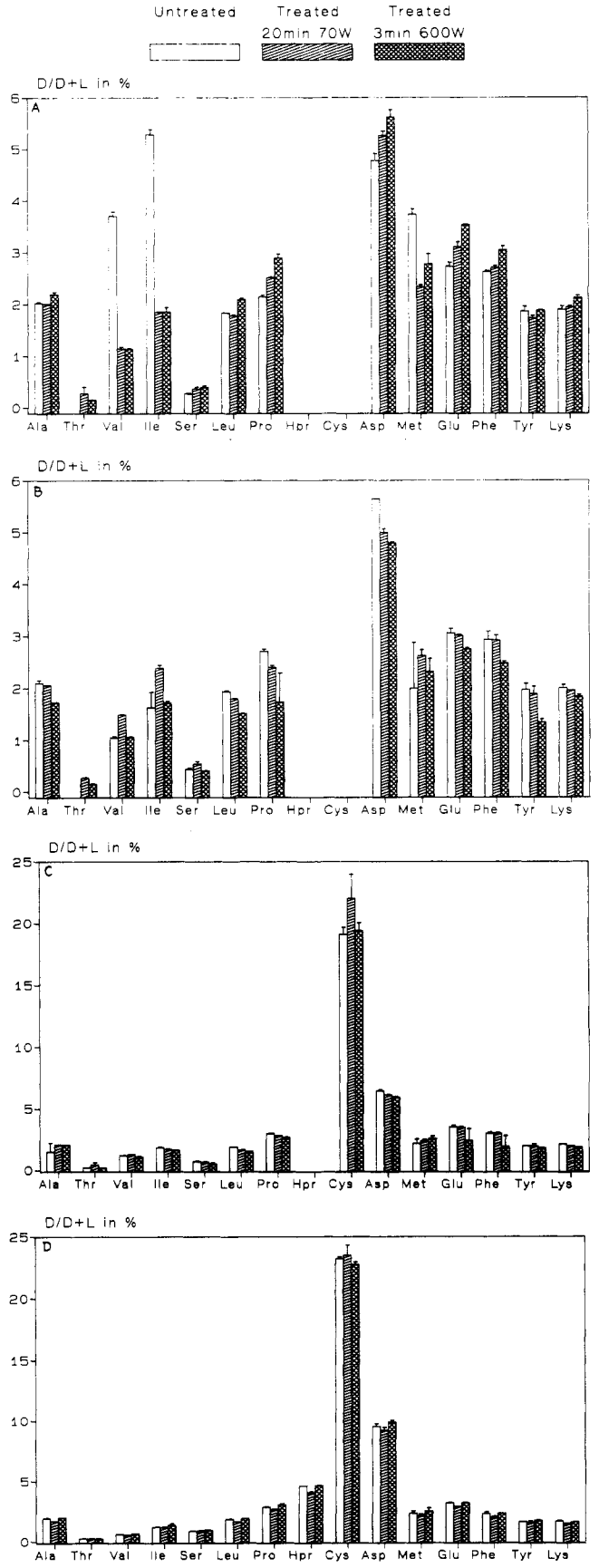

Figure 2. Amino acid isomerization in untreated and microwave-treated products after acid hydrolysis (segments on top of the bars are standard deviations): (A) UHT milk; (B) infant formula A; (C) infant formula B; (D) infant formula C.

gelatin. Similarly, D-Cys could only be detected in formulas B and C due to the relatively low yield of the derivatization method which includes the reduction of cystine

into cysteine (derivatized cystine cannot be analyzed on the Chirasil-Val column).

The extents of isomerization in untreated and treated products after acid hydrolysis are compared in Figure 2. As can be seen, for most amino acids, there is very little difference between untreated and treated samples. A noticeable exception is the reduced isomerization of Val and Ile in microwave-treated UHT milk when compared with the untreated sample. In the same sample, one can further observe slightly higher isomerization rates for Pro, Asp, Glu, and Phe. The same amino acids exhibit the opposite trend in formulas A and B. It can be further noticed that the isomerization of proline and hydroxyproline in formula C is not affected by the microwave treatment, in contradiction to the data reported by Lubec et al. (1989).

All of the variations apparently related to the microwave treatments are of the same order of magnitude as those observed among the untreated products (Figure 1). These small variations probably result from some heat-induced denaturation of the proteins, which in turn affects the isomerization rate of particular amino acid residues during the acid hydrolysis. Such effects have already been observed. For instance, methionine isomerization during the hydrolysis of α-albumin was dramatically reduced from 25% to 4% following a moderate alkaline treatment (Liardon and Ledermann, 1984). Such changes also occurred to a smaller extent for Val, Leu, and Pro in lysozyme and for Ile in bovine serum albumin following the same alkaline treatment (Liardon, unpublished results).

In view of these considerations, it can be concluded that under normal heating conditions (no excessive temperature or pressure buildup) microwave treatment does not induce significant isomerization of protein amino acids in milk and infant formulas.

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