JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Heat Markers and Quality Indexes of Industrially Heat-Treated [^{15}N] Milk Protein Measured in Rats

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To determine the bioavailability of industrially heat-treated milk proteins, male Wistar rats were given [^{15}N]-labeled meals containing either nonheated—micellar casein (CAS), milk soluble protein isolate (MSPI), and microfiltered milk (MF)—or heated products—"high temperature short time" pasteurized (HTST), "higher temperature, shorter time" pasteurized (HHST), ultrahigh temperature-treated (UHT), and spray-dried (SPRAY) milks. The postprandial distribution of dietary nitrogen was measured in the splanchnic area and urea. Digestibility was around 96% except for SPRAY (94%) and MSPI (98%). Ingested nitrogen recovered in the splanchnic bed was 19.3% for SPRAY, 16.7% for MF, and around 14−15% for other products. Deamination of dietary nitrogen reached 21.2, 20.6, and 18.2% of ingested nitrogen for MSPI, SPRAY, and CAS, respectively, and around 14−16% for other products. In our model, only spray drying led to a significant increase of splanchnic extraction. Moreover, the biological value of purified protein fractions appeared to be lower than that seen in products containing total milk protein.

KEYWORDS: Heat treatments; milk protein; protein quality; rat; in vivo assay

INTRODUCTION

Milk and milk products have constituted basic and important components of the human diet for centuries. Thermal processes, especially short-time treatments, are normally widely applied to these products to ensure their microbiological safety and to extend the shelf life of dairy products. Heat processing induces chemical and physical modifications that have a range of effects on their nutritional value and functional characteristics. The impact of heat treatments on the nutritional value of foods has been the subject of much debate in recent years. Experimental studies have mainly focused on the nutritional quality of milk proteins, soy-based infant formula, enteral formulas (*1*, *2*), legume proteins, and byproducts (*3*−*5*), assessed through chemical markers of heat damage (*6*) or rat growth bioassays (protein efficiency ratio or PER). These studies and others (*7*) have shown that heat processing induced chemical and physical changes, with various effects on the nutritional value and functional characteristics of food proteins. As for studies on milk protein quality, heat and residence time profiles have often proved excessive or unusual in the context of common industrial heat treatments. It has also been suggested that some chemical compounds, which formed during heat treatment, may have potential deleterious effects in animal models (*8*, *9*). Overall, despite the evidence of structural and functional modifications after heat treatments, little attention was paid to the biological value of heated milk protein.

During milk heating, protein modification and the formation of complexes between κ-casein and β-lactoglobulin (*10*) and between casein and lactose may adversely affect protein digestibility and postprandial nitrogen repletion due to the unavailability of some indispensable amino acids (IAAs). Lysinoalanine (LAL) formation and nonenzymatic browning are considered major factors limiting the nutritional value of conventionally heated dairy products. In the Maillard reaction, the formation of Amadori products due to the reaction of a reducing sugar (lactose) and the ϵ-NH_2 group of lysine—and possibly methionine—is likely to impair the bioavailability of these AAs by up to 30 or 40% and thus markedly reduces the biological value of the related protein. Moreover, although the presence of hydroxymethylfurfural and other Maillard products in ultra heat-treated (UHT) milk (with or without an excessive storage duration) has been fully explored (*11*, *12*), these studies rarely explored the physiological consequences of such compounds on dietary nitrogen disposal (*13*). As for the LAL content, a recent study performed on infant formulas (*14*)

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10.1021/jf051304d CCC: $33.50
Published on Web 01/21/2006

showed that since the 1980s, the chemical quality of commercial infant milks has much improved, because of more appropriate heat treatment procedures. Because lysine can undergo two independent processes—i.e., reaction with dehydroalanine residues resulting in LAL formation as well as the Maillard reaction—the content of available lysine has often been used as a marker of heat damage affecting dairy protein (*15*) and subsequently a marker of impaired protein quality.

However, a direct link between chemical modifications to essential AAs due to heat treatment and the in vivo bioavailability of protein has rarely been established. Moreover, to our knowledge, the true digestibility and postprandial retention of dairy protein nitrogen subjected to standard industrial heat treatments have never been studied in either animals or humans. We previously developed isotope methods to follow dietary nitrogen into the different body compartments of both rodent (*16*, *17*) and humans (*18*, *19*).

The aim of the present study was thus to explore in rats the fate of dietary nitrogen after the ingestion of dairy proteins subjected to standard industrial heat treatments. For this purpose, seven groups of 8−11 rats each acutely ingested a lactose-free mixed meal containing one of the following [^{15}N]-labeled milk products: three nonheat-treated products [micellar casein (CAS), milk soluble protein isolate (MSPI), and microfiltered milk (MF)] and four heat-treated milks ["high temperature short time" pasteurized (HTST), "higher temperature, shorter time" pasteurized (HHST), UHT, or spray-drying (SPRAY)]. Six hours after the ingestion of the experimental meal, the animals were sacrificed and dietary nitrogen was followed in some splanchnic tissues (liver, intestinal mucosa) and in both the plasma proteins and the body and urinary urea.

MATERIAL AND METHODS

[^{15}N]-Labeled Milk Products. Milk was [^{15}N]-labeled in the Unit of Milk Production (U.P.L., Unité de Production du Lait, INRA, Saint-Gilles, France) by giving 100 g/day of ($[^{15}NH_4)_2SO_4$ (12 atom % isotope enrichment, Euriso-top, Saint-Aubin, France) for 7 days to three lactating cows via a rumen fistula. The final isotopic enrichment of collected milk ranged from 0.6365 to 0.6682 atom % (AP). The milk was pooled and then defatted, and this skimmed [^{15}N]-labeled milk was subjected to a processing procedure shown schematically in **Figure 1**. Briefly, the skimmed milk was first microfiltered on a 1.4 μm membrane (GP7 Sterilox equipment), producing first a bacterial retentate and second microfiltered (MF) milk. Four different heat treatments were applied to this MF milk: HTST (72 °C, 20 s), HHST (96 °C, 5 s), UHT (140 °C, 5 s), and spray drying (Minor Spray pilot, Massy, France; inlet air temperature, 250 °C; outlet product temperature, 87 °C). All heat treatments were carried out using an Acti-joule equipment. The remnant bacterial retentate was diafiltered with osmosed water, and the permeate was concentrated and microfiltered on a 0.1 μm membrane (S7 pilot), thus allowing the separation of CAS in the retentate and milk soluble proteins in the permeate, the latter being concentrated further by ultrafiltration on a 5 kDa membrane (TIA apparatus). In this way, we obtained seven test products from the same initial milk sample. Moreover, heat-treated milks were all derived from the same MF. Because of the dramatic decrease in lactase activity after 3 weeks of age in rodents, all milks were lactose free, to avoid disruptions in to the digestive processes. Lactose was removed by ultrafiltration (polyethersulfone membrane cutoff at 10 kDa, Filtron apparatus, Amicon). All products were freeze-dried and stored at 4 °C to ensure correct conservation until their use for experiments.

Animals. Experiments were carried out in accordance with the guidelines of the French Committee for Animal Care and the European Convention for the Protection of Vertebrate Animals Used for Experimentation. Male Wistar rats (n = 70) (Harlan, Horst, The Netherlands), weighing 180−200 g at the beginning of the experiment, were housed in individual stainless steel screen-bottomed cages, in a room with controlled temperature and humidity (23 ± 1 °C; 50 ± 2% humidity) and a reverse 12 h light/dark cycle (light from 8:30 pm to 8:30 am). All animals were adapted for 14 days to an AIN-93M modified diet (*20*), containing 140 g of total milk protein per kg of food instead of casein plus cysteine (**Table 1**). Rats had access to the powdered diet according to a special feeding schedule of three meals per day. Between 8:30 and 8:45 am, rats were given a 6 g meal, and then for 2 × 60 min periods (12:00 midday−1:00 pm and 4:30 pm−5:30 pm), they had free access to food. Throughout the duration of the experiment, the rats had free access to water. This pattern was chosen in order to adapt the rats to prompt consumption of the experimental diet within a short period (15 min in the morning) while ensuring an adequate daily food intake (average 16 g/day/animal of dry food, i.e., 258 kJ/day/animal).

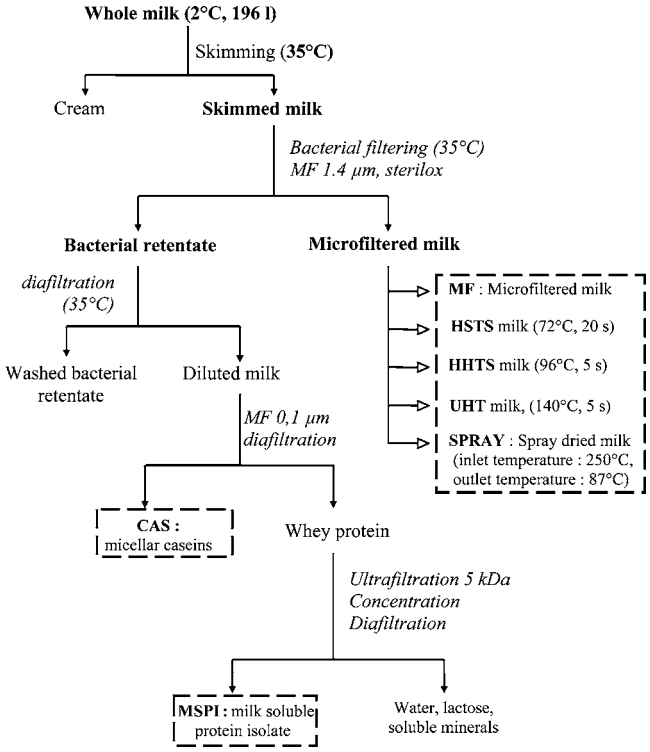

Figure 1. Processing and preparation of different heat-treated milks using [^{15}N]-labeled whole raw milk.

Table 1. Composition of the 14% Protein AIN-93M-Modified Diet (Adaptation Period)

	g/kg	% of energy
total milk protein (TMP)[a]	140	14.7
sucrose[b]	100	10.5
starch[c]	622.7	65.4
AIN-93M mineral mix[d]	10	
AIN-93M vitamin mix[d]	35	
soybean oil[e]	40	9.4
cellulose[f]	50	
choline[d]	2.3	
caloric content	15.9 kJ/g	

[a] Nutrinov (Rennes, France). [b] Eurosucre (Paris, France). [c] Cerestar (Haubourdin, France). [d] ICN Biomedicals (Costa Mesa, CA). [e] Bailly SA (Aulnay sous Bois, France). [f] Medias Filtrants Durieux (ZI Torcy, France).

Experimental Procedures and Sample Collection. On the morning of the 15th day, rats acutely ingested an experimental meal containing one of the seven [^{15}N]-labeled milk products: either a nonheat-treated product (CAS, MSPI, and MF) or a heat-treated product [HTST (72 °C, 20 s) or HHST (96 °C, 5 s), UHT (140 °C, 5 s), or spray drying (SPRAY)]. Experimental meals were isocaloric and prepared in order to mimic the composition of the AIN-93M modified diet containing

Table 2. Composition of the Experimental Diets (g/100 g)

	CAS	MSPI	MF	HSTS	HHTS	UHT	SPRAY
milk product	15.9	15	16	16	16	15.4	15.9
sucrose[a]	9.3	9	9.3	9.3	9.3	9.3	9.3
starch[b]	61.5	62	61	61	61	61.6	61.5
mineral mix[c]	3.1	3.1	3.1	3.1	3.1	3.1	3.1
vitamin mix[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
soybean oil[d]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
cellulose[e]	5.0	5.0	5.0	5.0	5.0	5.0	5.0
choline[c]	0.2	0.2	0.2	0.2	0.2	0.2	0.2

[a] Eurosucre. [b] Cerestar. [c] ICN Biomedicals. [d] Bailly SA. [e] Medias Filtrants Durieux.

14% milk protein (**Table 2**). Each meal contained the same levels of protein, carbohydrate, and fat.

At the beginning of the experimental procedure, absorbent paper was placed under the cages in order to collect urine during the 6 h following the experimental meal. The rats had access to the experimental meal for 30 min (8:30−9:00 am). The meal was then removed, and the animals had free access to water for 6 h. The rats were slaughtered 6 h after ingestion of the [^{15}N]-labeled meals. They were anesthetized with sodium pentobarbital (200 mg/kg body weight) and injected with 5000 IU heparin per animal (Laboratoires Choay, Gentilly, France). After incision of the abdomen, blood was withdrawn from the abdominal cavity after rupture of both the caudal vena cava and the aorta. The gastric, intestinal, and cecal contents were sampled and stored at −20 °C. The small intestine wall was divided into three equal parts (proximal, medial, and distal) and weighed and stored at −20 °C until analysis, together with the liver. Blood was ice-stored in individual glass tubes and centrifuged (20 min, 3000*g*, 4 °C). The plasma was stored at −20 °C for subsequent analysis. The bladder was removed, incised, and emptied of urine. The absorbent paper and bladder were rinsed with distilled water, and the eluates from each rat were pooled and stored at −20 °C until analysis, as previously described (*16*). The gut contents were weighed after freeze drying. The liver, gut contents, and small intestine wall sections were freeze-dried and crushed for subsequent isotopic analysis.

Analytical Methods. *Biochemistry of Milk Products.* The lactulose content of the powder was determined according to the International Dairy Federation method (*21*). Alkaline phosphatase activity was determined according to the International Dairy Federation method (*22*). The acid hydrolysis of protein was performed under a vacuum in the presence of 6 N HCl for 24, 48, and 92 h at 110 °C using an AA analyzer (Pharmacia alpha plus 2) according to the method described by Spackman, Stein, and Moore (*23*).

Analyses were performed by reverse phase high-performance liquid chromatography (RP-HPLC) using a Vydac C4 column (150 mm × 4.6 mm i.d.). Separation was achieved at a flow rate of 1 mL/min and at 40 °C using a linear gradient with acetonitrile. Solvent A contained 0.106% (v/v) trifluoroacetic acid (TFA) in MilliQ water, and solvent B contained 0.1% (v/v) TFA in acetonitrile−milliQ water (80/20 v/v). For the analyses of whole proteins, lactose-free milk powders were reconstituted from freeze-dried milk powders by adding deionized water to a final concentration of 3% (w/v), corresponding to 28 g/kg. These samples were then reduced by a 5-fold dilution with 0.1 M Tris-HCl, pH 8, buffer containing 8 M urea and 12.5 mM dithiotreitritol. After standing at 37 °C for 1 h, samples were further diluted with 0.2% TFA to give approximately 1 g/L protein solution at pH 2.2. Separation was performed with a linear gradient from 37 to 62% with eluent B for 58 min, followed by detection at 214 nm.

Milk soluble proteins were obtained after the precipitation of lactose-free milk powders (3%) with 1 N HCl at pH 4.6. Caseins and denatured milk soluble proteins were removed by centrifugation at 2500*g* for 15 min. Supernatants diluted 12.5-fold in eluent A were analyzed by RP-HPLC under the elution conditions described above, with a linear gradient from 46 to 61% applied for 15 min. For MSPI, powder was solubilized at 6% in deionized water and diluted 100-fold in eluant A for RP-HPLC.

Determination of Lactosylation Rates for β-Lactoglobulin (β-Lg) Originating from SPRAY Milk. Whey proteins from SPRAY milk powder were obtained as described above. Analysis of the specific lactosylation of β-Lg was performed using on-line RP-HPLC coupled with an electrospray ionization mass spectrometry (ESI-MS) device. RP-HPLC conditions were identical to those previously described. Mass was determined using an API-III Plus mass spectrometer (Sciex, Thornhill, Ontario, Canada) equipped with an atmospheric pressure ionization source. Sample analyses were carried out as previously described in the literature (*24*). Molecular masses were determined from the measured *m*/*z* values, and the mass assignments of β-Lg forms were confirmed by AA sequence determination using tandem mass spectrometry (MS-MS) analysis, as previously described (*24*).

Biological Samples. Plasma urea concentrations were determined by an enzymatic assay using urease and the reaction of ammonia with hypochlorite and phenol to form blue indophenol (Urea Nitrogen endpoint kit, Sigma-Aldrich Chimie, Saint-Quentin Fallavier, France). Urinary urea assays were performed using the urease reaction (Urea kit, bioMerieux, Marcy l'Etoile, France) on a Mascott + 3× spectrophotometer (Lisabio, Pouilly en-Auxois, France).

Plasma proteins were precipitated using 50 μL of a 1 g/mL sulfosalicylic acid solution per mL of plasma. Samples were therefore stored at 4 °C for 1 h and centrifuged (4 °C, 20 min, 3000*g*); the supernatant was retained for the isolation of plasma urea. The pellets were frozen at −20 °C and then freeze-dried.

Urinary and plasma urea were isolated on a Na/K form of a cation exchange resin (Biorad Dowex AG50-X8, mesh 100−200, Interchim, Montluçon, France) as previously described (*25*). The resin-containing, urea-derived NH_4^+ was stored at 4 °C until isotopic determination, prior to which the resins were eluted with a 2.5 mmol/L KH_2SO_4 solution.

Nitrogen Contents and Isotopic Determinations. Nitrogen percentages and [^{15}N] enrichments were measured using isotopic ratio mass spectrometry (Optima, Fisons Instruments, Manchester, United Kingdom) coupled with an elemental analyzer (NA 1500 series 2, Fisons Instruments) with atropina (Carlo Erba Instruments, Fisons, Arcueil, France) as the standard, as previously described (*26*). Briefly, aliquots (solid: freeze-dried digesta, intestinal mucosa, or liver samples; liquid: urine and plasma) were burned in the elemental analyzer. CO_2 and water were trapped, and the isotope ratio in N_2 was measured using a calibrated nitrogen gas as the $^{15}N/^{14}N$ reference. The AP and the atom percent excess (APE) were then calculated.

Calculations. The exogenous nitrogen ($N_{exo-mmol}$) present in samples was calculated as follows:

$$N_{exo-mmol} = N_{tot-mmol} \times (APE_s/APE_m)$$

where $N_{tot-mmol}$ is the amount of total nitrogen (N) in the sample; APE_s is the [^{15}N]-enrichment excess of the sample = AP of the sample − basal enrichment of the sample; and APE_m is the [^{15}N]-enrichment excess of the meal = AP of the meal − basal enrichment of the sample.

The exogenous nitrogen recovered in the urea body pool was calculated assuming that urea was uniformly distributed throughout the total body water and according to the following formula:

$$N_{exo-urea} = C_{urea} \times 2 \times 0.67/0.92 \times BW \times (APE_{urea-s}/APE_m)$$

where C_{urea} is the concentration of urea in the plasma; BW is body weight; APE_{urea-s} is the [^{15}N]-enrichment excess of urea in the sample; and APE_m is the [^{15}N]-enrichment excess of the meal.

In the rat, the mean percentages of body water and of water in plasma are assumed to be, respectively, 67 and 92% (*27*). The total amount of exogenous nitrogen transferred to urea is the sum of the exogenous nitrogen excreted in urinary urea and the exogenous nitrogen recovered in the body urea pool. The exogenous nitrogen recovered in plasma proteins was calculated assuming that plasma represents 3.5% of BW (*28*).

Results, when expressed as the percentage of ingested nitrogen ($N_{\%ing}$) recovered in tissues, plasma, or urine samples, were calculated as follows:

$$N_{\%ing} = N_{exo-tot}/N_m \times 100$$

Table 3. AA Composition of the Milk Products (%)

	CAS	MSPI	MF	HTST	HHTS	UHT	SPRAY
Asp	6.59	10.71	7.44	7.52	7.58	7.59	7.71
Thr	3.71	4.78	4.12	4.24	4.27	4.38	4.44
Ser	4.33	3.64	5.05	5.03	5.12	5.11	5.10
Glu	22.14	17.05	19.56	19.66	19.75	20.08	19.69
Pro	10.30	6.86	11.25	11.18	8.76	8.29	7.10
Gly	1.44	1.66	1.59	1.61	1.57	1.63	1.66
Ala	2.37	4.16	2.97	2.92	2.99	3.06	3.08
Cys	–	2.91	0.87	0.75	0.83	0.80	0.94
Val	6.28	5.41	6.06	5.79	6.02	5.99	6.49
Met	2.78	2.18	2.32	2.18	2.26	2.12	2.54
Ile	4.94	5.51	4.83	4.77	5.16	5.21	4.92
Leu	9.06	11.85	8.84	9.16	9.45	9.70	9.57
Tyr	5.77	3.85	5.29	5.45	5.69	5.71	5.66
Phe	5.36	4.16	4.85	5.08	5.31	5.15	5.16
His	4.63	2.29	3.02	2.94	3.08	3.12	3.17
Lys	6.28	9.98	8.03	7.90	8.19	8.10	8.48
Arg	4.02	3.01	3.90	3.83	3.97	3.94	4.30
IAA	43.05	46.15	42.08	42.07	43.73	43.78	44.76
DAA[a]	56.95	53.85	57.92	57.93	56.27	56.22	55.24
BCAA	20.29	22.77	19.73	19.73	20.63	20.90	20.97

[a] DAA, dispensable amino acids.

where $N_{exo-tot}$ is the total amount of dietary nitrogen recovered in tissues, plasma, or urine; and N_m is the total amount of ingested N from the meal.

Orocecal protein digestibility (DIG) was calculated as the difference between the nitrogen ingested and the nitrogen recovered in the gastrointestinal tract after 6 h, divided by ingested nitrogen.

Net postprandial protein utilization (NPPU) was calculated as the percentage of ingested nitrogen that was retained in the body: NPPU = DIG − $N_{\%ing-urea}$. Postprandial biological value (PBV) was calculated as the percentage of absorbed nitrogen retained in the body: PBV = 100 − $N_{\%ing-urea}$.

Statistical Analysis. Values are presented as means ± standard errors. Means and standard errors were calculated using the MEANS procedure of the SAS/STAT software (version 6.11, Cary, NC). Statistical comparisons of the different meal groups were performed using the SAS/STAT software (version 6.11, Cary, NC). A one-way analysis of variance was performed using the GLM procedure of SAS, and Bonferoni's posthoc tests were used for pairwise comparisons. The contrast statement was used to compare on one hand heated vs nonheated products and on the other hand purified milk proteins (CAS and MSPI) vs total native milk proteins. An a posteriori analysis of our results led us to consider SPRAY apart from other heated products. The rationale for this approach was the specific recovery of lactosylation in soluble protein from SPRAY. Differences were considered to be significant when the p value was <0.05.

RESULTS

Microbiological and Biochemical Characteristics of Milk Products. *Microbiological Characteristics.* All products were tested for microbiological flora. All products except SPRAY and CAS exhibited a total flora level and a coliform level <10 colony-forming units (cfu)/g. The total flora was 5×10^2 cfu/g for CAS and 2.1×10^4 cfu/g for SPRAY.

*AA Composition (**Table 3**).* With respect to the proportions of individual AAs in different milk proteins, the main differences were observed between MSPI and CAS products. Asp, Ala, Ile, Leu, and Lys levels were higher in MSPI than in CAS. On the other hand, Ser, Glu, Pro, Tyr, Phe, His, and Arg levels were higher in CAS. Consequently, the proportion of branched chain amino acids (BCAA) and IAAs was higher in the MSPI group than in the CAS group. AA proportions in MF, HSTS, CAS, HHTS, and UHT products were similar, except for the Pro content that seemed to decrease with heating. Finally, MSPI proteins exhibited the highest levels of IAAs and BCAAs, followed by the SPRAY proteins.

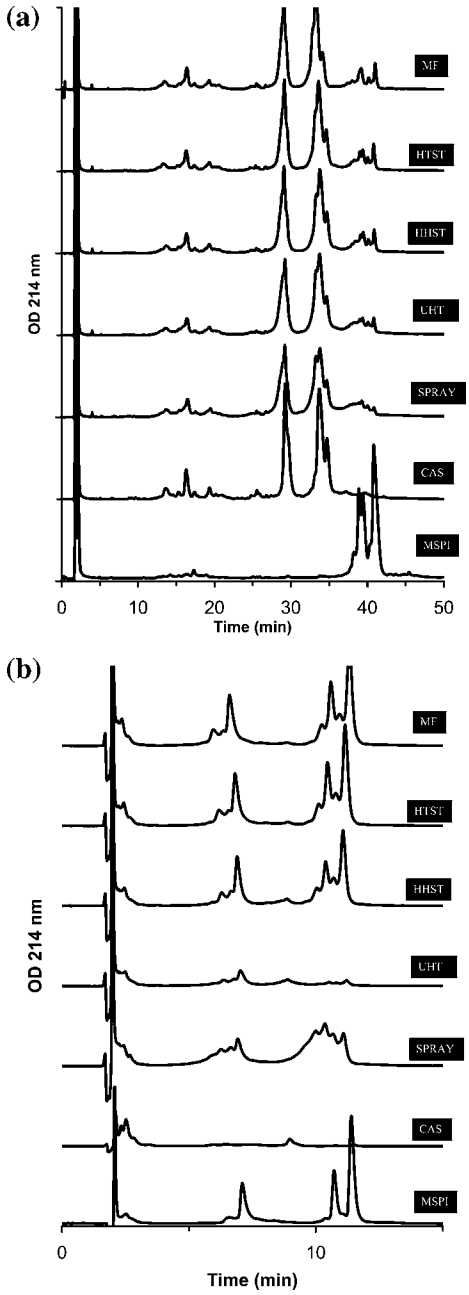

Figure 2. HPLC profiles of different milk products. (**a**) Whole protein profiles (pH 2.2) and (**b**) soluble fraction (pH 4.6).

*HPLC Profiles of Milk Products and Mass Spectrum of β-Lg from Spray-Dried Milk (SPRAY) (**Figure 2**).* The RP-HPLC profiles of the different lactose-free powders highlighted the composition in whole proteins. As compared with MF, the profile of the nonsoluble protein fraction (**Figure 2a**) did not change as a result of heat treatment, unlike that of soluble proteins. As heat treatment intensified, individual separations of soluble proteins became more difficult, as their peaks grew broader (**Figure 2b**). To better characterize soluble proteins, caseins were precipitated at pH 4.6 and the supernatants were analyzed by RP-HPLC. The RP-HPLC profile of soluble proteins arising from MF was strictly similar to that of MSPI, which corresponded to the milk soluble proteins. The profiles following HTST and HHST treatments exhibited a slight decrease in the α-La and β-Lg peaks. Proteins had almost

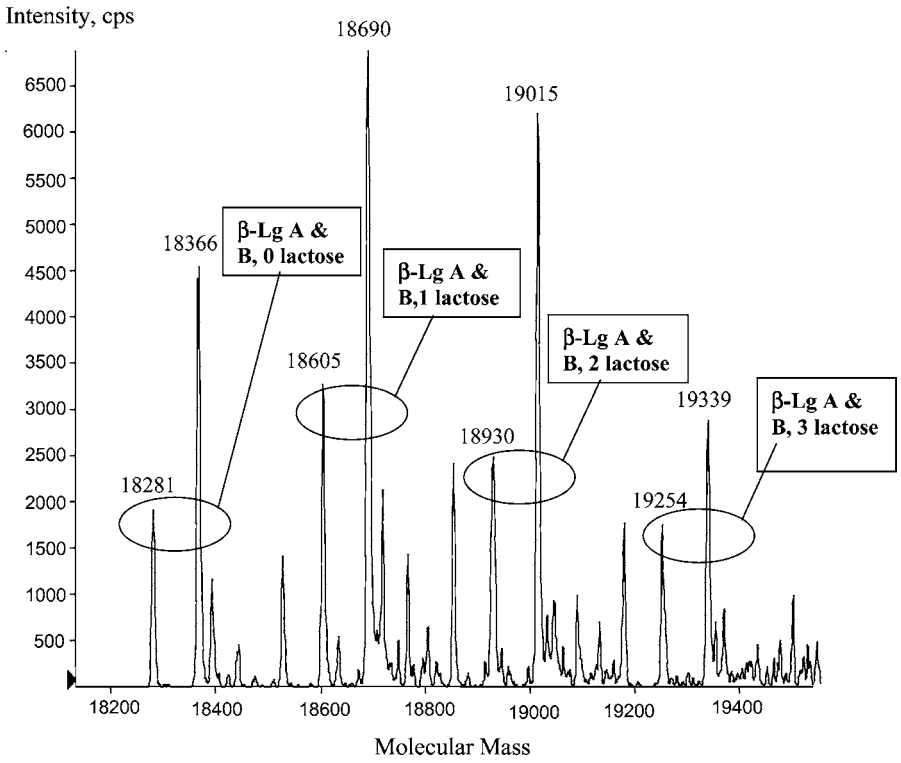

Figure 3. Reconstructed spectrum of β-Lg from spray-dried whey protein. Original β-Lg A and β-Lg B (0 lactose), 18366 and 18281, respectively. β-Lg A and β-Lg B with one lactose, 18690 and 18605, respectively. β-Lg A and β-Lg B with two lactose molecules, 19015 and 18930, respectively. β-Lg A and β-Lg B with three lactose molecules, 19339 and 19254, respectively.

Table 4. Ingested Nitrogen and Residual Dietary Nitrogen in the Gastrointestinal Tract 6 h after the Meals[a]

		dietary nitrogen in the gastrointestinal tract 6 h after the meal							
	ingested nitrogen[b]	stomach		small intestine		cecum		total	
	(mmol)	μmol	% ingested N	μmol	% ingested N	μmol[b]	% ingested N[b]	μmol[b]	% ingested N[b]
CAS	6.97 ± 0.93 a	12 ± 8	0.17 ± 0.12	49 ± 40	0.66 ± 0.46	228 ± 94 b	3.35 ± 1.27 b	290 ± 103 b	4.16 ± 1.22 a
MSPI	6.93 ± 1.91 a	11 ± 10	0.16 ± 0.11	27 ± 9	0.39 ± 0.07	82 ± 41 a	1.20 ± 0.56 a	121 ± 47 a	1.74 ± 0.56 b
MF	5.56 ± 1.52	6 ± 1	0.12 ± 0.02	28 ± 11	0.58 ± 0.22	157 ± 55	3.13 ± 0.53 b	191 ± 59	3.77 ± 0.42 a,c
HTST	6.85 ± 0.99 a	10 ± 8	0.15 ± 0.12	31 ± 14	0.45 ± 0.18	197 ± 61 b	2.91 ± 0.84 b	238 ± 72 b	3.49 ± 0.99 a,c
HHST	5.24 ± 1.49	9 ± 12	0.17 ± 0.20	30 ± 17	0.59 ± 0.35	159 ± 57	3.26 ± 0.87 b	198 ± 62	3.99 ± 0.88 a,c
UHT	5.96 ± 1.19	7 ± 4	0.12 ± 0.07	36 ± 20	0.61 ± 0.33	199 ± 55 b	3.29 ± 0.52 b	242 ± 60 b	4.00 ± 0.63 a,c
SPRAY	4.68 ± 1.01 b	9 ± 6	0.19 ± 0.10	30 ± 12	0.64 ± 0.19	221 ± 64 b	4.70 ± 0.66 c	260 ± 78 b	5.53 ± 0.85 a,d

[a] Values are means ± SE. Values with different letters are statistically different, and values with the same letter are not statistically different; a value with no letter does not differ from any other value; that is, for dietary nitrogen in the cecum 6 h after the meal (expressed as % of ingested nitrogen), MSPI differs from all other groups and SPRAY differs from all other groups except CAS. [b] Significant effect of test meal ($p < 0.05$).

disappeared from the soluble fraction at pH 4.6 following UHT treatment, while major impairment of the HPLC profile was observed following SPRAY treatment. **Figure 3** shows the reconstructed mass spectrum of β-Lg from SPRAY. Peaks represent different real masses corresponding to the original β-Lg genetic variants (β-Lg A and B) and its modified—i.e., lactosylated—forms (addition of one, two, or three molecules of lactose). Around 80% of β-Lg A and B was modified by the covalent binding of one or more lactose residues.

Animals. During the adaptation period, the growth curves of all rats were regular and similar (data not shown). On the experimental day, no differences were observed between groups in terms of body, liver, and kidney weights, which were, respectively, 243.1 ± 12.7, 7.8 ± 0.7, and 1.6 ± 0.1 g (data not shown). After the 15 min experimental meal, rats that were offered the MSPI-, CAS-, or HTST-based meals ingested significantly more dietary nitrogen (around 6.9 mmol) than rats offered SPRAY-based meal (4.7 mmol) (**Table 4**).

Digestive Data and Postprandial Distribution of Dietary N. *Dietary Nitrogen Recovered in the Gastrointestinal Tract 6 h after the Meal (**Table 4**).* The percentage of ingested nitrogen remaining in the stomach 6 h after ingesting the experimental meals was low and ranged, respectively, from 0.12 (MF and UHT) to 0.19% (SPRAY). In the intestinal lumen, dietary nitrogen levels ranged from 0.39 (MSPI) to 0.66% (CAS) of ingested nitrogen. Because of high interindividual variability, there were no significant differences between meal groups concerning these parameters. Dietary nitrogen levels in the cecal compartment were about 4−5 times higher than in the two other digestive compartments taken together, except for the MSPI group (2-fold). The amounts of ingested nitrogen recovered in the cecum were lowest in the MSPI group (82 μmol) and highest in the CAS and SPRAY groups (228 and 221 μmol, respectively). Cecal dietary nitrogen recovered in the MSPI group was statistically and significantly different from that observed in the CAS, HTST, UHT, and SPRAY groups. Finally, statistics

Table 5. Transfer of Dietary Nitrogen to the Urea Pool and Splanchnic Sequestration 6 h after the Ingestion of the Experimental Meals (Expressed as % of Ingested Nitrogen)[a]

	CAS	MSPI	MF	HSTS	HHTS	UHT	SPRAY
				deamination			
body urea pool[b]	4.10 ± 1.57 a	4.49 ± 1.13	4.08 ± 1.56 a	3.29 ± 1.02 a	4.57 ± 1.48	3.73 ± 0.56 a	6.02 ± 0.70 b
urinary urea pool[b]	14.08 ± 2.08	16.72 ± 4.62 a	10.24 ± 1.36 b	11.38 ± 4.11 b	11.89 ± 1.90 b	10.68 ± 3.70 b	14.61 ± 3.76
total deamination[b]	**18.19 ± 3.15**	**21.20 ± 5.38** a	**14.32 ± 2.33** b	**14.67 ± 4.03** b	**16.45 ± 2.04**	**14.41 ± 3.58** b	**20.63 ± 3.78** a
				splanchnic area			
intestinal mucosa							
proximal[b]	1.76 ± 0.47 a	1.90 ± 0.38	2.33 ± 0.63	1.79 ± 0.32	2.15 ± 0.48	1.90 ± 0.51	2.46 ± 0.67 b
medial[b]	1.67 ± 0.24 a	1.70 ± 0.23 a	1.57 ± 0.50 a	1.37 ± 0.32 a	1.68 ± 0.32 a	1.34 ± 0.29 a	2.14 ± 0.29 b
distal	0.74 ± 0.15	0.79 ± 0.22	0.74 ± 0.37	0.67 ± 0.31	0.74 ± 0.36	0.64 ± 0.16	0.87 ± 0.14
total intestinal mucosa[b]	***4.17* ± *0.71*** a	***4.40* ± *0.59*** a	***4.64* ± *0.78***	***3.79* ± *0.68*** a	***4.70* ± *0.69***	***3.89* ± *0.69*** a	***5.64* ± *0.74*** b
liver[b]	***6.42* ± *1.06*** a	***7.21* ± *0.98*** a	***7.56* ± *1.09***	***6.18* ± *0.94*** a	***6.23* ± *1.27*** a	***6.72* ± *0.86*** a	***8.77* ± *0.65*** b
plasma proteins[b]	***3.70* ± *0.62*** a,d	***3.64* ± *0.42*** a,d	***4.51* ± *0.57*** a,c	***3.71* ± *0.23*** a,d	***3.96* ± *0.38*** a	***4.11* ± *0.42*** a	***5.43* ± *0.49*** b
total splanchnic area[b]	**14.33 ± 2.16** a	**15.24 ± 1.55** a	**16.71 ± 2.15** a	**14.10 ± 1.21** a	**15.14 ± 1.79** a	**14.96 ± 1.60** a	**19.28 ± 2.03** b

[a] Values are means ± SE. Values with different letters are statistically different, and a value with no letter does not differ from any other value; that is, for plasma proteins, the SPRAY group differs from CAS, MSPI, MF, HSTS, HHTS, and UHT groups and the MF group differ from CAS and HSTS groups, but the MF group does not differ from HHTS. [b] Significant effect of test meal ($p < 0.02$).

Table 6. Chemical Markers of Heat Treatment and Indexes of Protein Quality Measured in Vivo[a]

	chemical heat marker contents			in vivo indexes of protein quality		
milk product	soluble protein peak area[b]	PAL (mU/100 g)	lactulose (mg/100 g)	digestibility[c] (%)	BV[c] (%)	NPPU[c] (%)
CAS		413321	none	95.63 ± 1.2 a	81.81 ± 3.15	77.44 ± 3.97
MSPI		240604	none	98.30 ± 0.52 b	78.80 ± 5.38 a	77.01 ± 5.22
MF	100	289199	176	96.23 ± 0.42 a,c	86.01 ± 2.49 b	82.24 ± 2.58 a
HSTS	98	4525	167	96.28 ± 0.73 a,c	85.33 ± 4.03 b	81.61 ± 3.67 a
HHTS	82	15	166	95.85 ± 0.81 a,c	83.67 ± 2.14	79.53 ± 2.14
UHT	22	none	361	96.00 ± 0.63 a,c	85.59 ± 3.58 b	81.35 ± 3.90 a
SPRAY	105	137221	140	94.60 ± 0.79 a	79.37 ± 3.78 a	73.96 ± 3.91 b

[a] Values with different letters within a same column are statistically different, and a value with no letter is not different from any other value; that is, for BV values, CAS and HHTS are not different from any other value, and MF, HSTS and UHT are significantly higher than SPRAY and MSPI. [b] In percentage of the reference product (MF). [c] Significant effect of the test meal ($p < 0.005$).

regarding dietary nitrogen recovered from the entire gastrointestinal tract 6 h after the meal produced results similar to those obtained in the cecal compartment.

Postprandial Distribution of Dietary N. **Table 5** shows the percentage of dietary nitrogen retained in the splanchnic organs and transferred to body and urinary urea (deamination pool). The meal had a global effect on nitrogen retention in the proximal portion of the intestinal mucosa, with a statistically significant difference between CAS and SPRAY groups (Bonferoni's posthoc test). In the medial mucosa, a significantly higher retention of dietary nitrogen was observed in the SPRAY group than in any other groups. There was no statistical effect of the meal on the amount of dietary nitrogen retained in the distal intestinal mucosa. As for the total intestinal mucosa, the percentage of dietary nitrogen recovered in the SPRAY group was significantly higher than in the CAS, MSPI, HSTS, and UHT groups.

The hepatic dietary nitrogen content was 1.3−1.5 times higher in the SPRAY group than in any of the other groups except for MF, and this difference was statistically significant. The amount of hepatic dietary nitrogen recovered in the MF group tended to be higher than in other groups but without attaining statistical significance. Similarly, levels of dietary nitrogen incorporated into plasma proteins were significantly higher in the SPRAY group than in any other groups (1.4−1.5-fold). Moreover, this parameter was significantly higher in MF rats than in CAS, MSPI, and HSTS animals. Finally, the retention of dietary nitrogen in splanchnic proteins reached the highest levels in the SPRAY group (19% of ingested nitrogen) and differed significantly ($p < 0.0001$) from that observed in other groups (14−17%).

Deamination. Dietary nitrogen recovered in the body urea pool 6 h after the meal was significantly higher in the SPRAY group than in the HSTS group. Irreversible transfer of dietary nitrogen to the urinary urea pool was higher in the MSPI group than in the MF and UHT groups. Finally, total deamination reached its highest levels in SPRAY and MSPI animals (about 21% of ingested nitrogen), followed by the CAS group (18%) and then by other milk products, where deamination ranged from 14 to 16%.

Contrast analysis underlined a difference between purified proteins (MSPI and CAS) and milk products with the exception of SPRAY ($p < 0.05$). The contrast between heated (HSTS, HHTS, UHT, and SPRAY) and nonheated products was not significant.

Biochemical Heat Markers and Nutritional Quality Indexes (Table 6). *Heat Markers.* Biochemical heat markers include the peak area of HPLC profile at pH 4.6, lactulose, and phosphatase alkaline (PAL) contents. Analyses of heat markers performed on skimmed heat-treated milks demonstrated some differences between HSTS, HHTS, and UHT milks on one hand and SPRAY milk on the other hand.

Table 6 shows that, as compared to MF, the peak areas of RP-HPLC profiles at pH 4.6 decreased by 19% with HHST

and 78% with UHT. The soluble protein fraction of SPRAY was modified but not lost. The lactulose content was 2-fold higher in UHT milk than in SPRAY milk (361 vs 140 mg/100 g). The PAL content was highest in MF milk (289199 mU/100 g), followed by SPRAY milk (137221 mU/100 g) and dropped dramatically in pasteurized milks, whereas the PAL test was negative in UHT milk. As PAL is concentrated during processes to isolate CAS and milk soluble proteins (MSPI), the results regarding these two products were not comparable with one another and of course with those for products derived from MF.

In vivo indexes of protein quality exhibited some statistical differences between products. Because of the differences observed in the recovery of total dietary nitrogen in the cecum, orocecal digestibility was noticeably influenced by the meal ($p < 0.0001$). The MSPI group exhibited a particularly high level of nitrogen digestibility (98.3 ± 0.5%) and the SPRAY group the lowest level (94.6 ± 0.8%), whereas digestibility ranged from 95.6 (CAS) to 96.2% (HSTS and MF) in the other groups. Thus, the orocecal digestibility of MSPI and SPRAY nitrogen differed to a statistically significant extent from all other orocecal digestibilities (except that of CAS nitrogen for the latter) and from each other.

NPPU represents the fraction of ingested nitrogen retained in the body. The SPRAY group exhibited the lowest NPPU (74.0%) followed by MSPI and CAS (77.0 and 77.4%, respectively), while other milk product groups showed NPPU values ranging from 79.5 (HHTS) to 82.2% (MF). The biological values (BVs) of MSPI and SPRAY were the lowest (79%), and the BV of MF was the highest (86%).

As for deamination, statistical contrast analysis of NPPU and BV underlined a difference between purified proteins (MSPI and CAS) and milk products except for SPRAY. The contrast between heated (HSTS, HHTS, UHT, and SPRAY) and non-heated products was not significant.

DISCUSSION

This study focused on the impact of thermal treatment on the bioavailibility of dairy proteins. The originality of this work consisted in the fact that we assessed in vivo the protein quality of dairy products with well-controlled and defined biochemical characteristics and technological processes. First, microfiltration processing allowed us to obtain native caseins in a micellar form as in milk, instead of the caseinates that are most commonly tested. To our knowledge, there have been no other reports examining nitrogen bioavailibility originating from casein in a native form. Second, all heat-treated milk products were obtained from the same reference milk. In addition, the proteins in these products were similar to those originating from commercially available heat-treated milks. When tested on a rat model, we found that only one heat treatment, SPRAY drying, resulted in a significant modification of postprandial nitrogen metabolism. Moreover, purified protein fractions and particularly the milk soluble proteins exhibited lower postprandial retentions and BVs than total milk proteins obtained from milk products.

We have explored different biochemical markers to assess the degree of denaturation or modification of milk proteins. These markers concerned HPLC profiles, AAs, lactulose, and PAL. We observed a gradual disappearance of milk soluble proteins in HTST, HHST, and UHT, due to their denaturation during heat treatment. Structural and functional modifications of milk proteins following heating have been extensively documented during the past 40 years (*6*, *29*−*32*), although only a few data regarding HTST and HHTS processes are currently available. It is known that native milk soluble proteins are soluble at their isoelectric point (pH ∼5.3−4.6) while denatured milk soluble proteins are insoluble. Regarding the spray-dried product, milk soluble proteins were not denatured but chemically modified; otherwise, they would have precipitated at pH 4.6. This had already been observed and extensively studied in whey powder, especially when β-Lg was specifically modified by the covalent binding of a lactose residue during the early stages of the Maillard reaction (*24*). Consequently, unlike HTST, HHST, and UHT products, we considered that milk soluble proteins in the SPRAY had not been denatured but modified by the formation of a complex between β-Lg and at least one molecule of lactose. Bouhallab et al. (*33*) reported that the solubility of lactose−β-Lg conjugates at pH 4.6 was increased when compared with unmodified β-Lg and was proportional to the number of lactosylated sites, thus explaining why the largest peak area was observed for SPRAY. As discussed below, our study linked a modification of postprandial metabolism with protein lactosylation but not with protein denaturation due to heating.

The true orocecal digestibility of milk products was about 95−96%, which was similar to the values already reported in the literature for milk protein. In humans, the true ileal and fecal digestibilities of total milk protein have been reported to be 95 and 96%, respectively (*18*, *25*). During our rat study, heat treatment did not impair the digestibility of dairy proteins with the exception of spray drying, which slightly (1.5%) but significantly reduced the coefficient of absorption. Losses of digestibility following heat treatment have been observed during other studies under harsh temperature conditions. For example, Gilani et al. (*13*) reported in rats a decrease in true fecal digestibility of 15−27% when either lactalbumin or skimmed milk powder was heated at 121 °C for 1 h. A heat/alkaline treatment (6 h at 65 °C) reduced the real ileal digestibilities of casein and β-lactoglobulin from 93 to 85% and from 97 to 87%, respectively, in pigs (*34*). Few studies have addressed standard industrial treatments such as UHT and pasteurization. No impairing effect of these treatments on digestibility has been reported in rats (*11*) or pigs (*35*). The sterilization of liquid infant formula was reported to diminish the protein digestibility in vitro (*36*) as well as in rats (*37*) when compared to spray drying or UHT. Another interesting result of our study was the high orocecal digestibility of MSPI. As reported before, de Vrese et al. (*34*) found that β-Lg ileal digestibility in pigs (97%) was 4% higher than that of casein but without the difference reaching a level of significance. The true ileal digestibility of individual AAs in milk soluble protein and sodium or calcium caseinates has been investigated in pig and rat models, without noticeable differences (*38*). We cannot exclude that the higher digestibility of MSPI reported during our study is a methodological artifact. Indeed, we hypothesized that the amount of dietary nitrogen 6 h after the meal would be negligible in the colon. Because milk soluble proteins are more rapidly delivered in the intestine than casein (*39*−*41*), more dietary nitrogen may have been present in the colon in the MSPI group than in other groups 6 h after the meal and our result regarding MSPI digestibility may be overestimated.

Surprisingly, the present study did not demonstrate any correlation between the temperature applied to proteins and their nitrogen availability, despite the fact that some studies have shown significant biochemical alterations to conventionally heat-treated milks (*11*, *42*) and despite the denaturation of the milk soluble protein fractions that we evidenced. However, to our knowledge, studies with [^{15}N]-labeled meals based on dairy

industrial products have never been performed to date. The transfer of dietary nitrogen to body and urinary urea was similar with MF, HTST, and UHT milks—around 14% of ingested nitrogen—and slightly higher with HHST milk (16% of ingested nitrogen). In line with this, we previously reported a postprandial deamination of milk protein ranging from 16 to 25% depending on meal composition and the habitual protein intake (*25*, *43*, *44*). In rats adapted to a normal protein diet, we found a postprandial deamination of 8% (*17*). This value was lower than that reported in the present study, but it was obtained 5 h after the meal and the rats were adapted to a small morning meal of 3 g, instead of 6 g in this study. In our rat model, only atomization led to a significant increase of oxidative losses, indicating that parameters other than a high temperature applied to milk protein may affect their nutritional quality. In the case of SPRAY, a combination of temperature and water stream may have resulted in a modification to those proteins—and not a denaturation—and markers other than those for heat treatment should be investigated. In particular, the degree of milk protein lactosylation appears to be important because it is known to be responsible for lysine blockage. A low level of lysine availability increases postprandial oxidative losses (*45*). However, because the SPRAY product was not prepared in the same technical conditions than industrial milk powders and because it was bacterially contaminated during drying, it may not be representative of commercially available products.

Although we were able to evidence a modification of postprandial nitrogen metabolism with SPRAY, this was not the case with UHT, despite a marked denaturation of the soluble protein fraction. UHT treatment causes major denaturation of whey proteins, mainly through the aggregation of β-Lg to α_{S2}- and κ-casein and the formation of primary aggregates of β-Lg and α-lactalbumin (α-Lac) (*46*, *47*). One hypothesis is that denaturation did not sufficiently affect the bioavailability of AAs (especially lysine) to result in increased oxidation over a 6 h period. Nor can we exclude a lack of sensitivity of our rat model particularly because no kinetic data were collected. Some other arguments can be opposed to the utilization of a rat model, in relation with its size. Amounts of ingested N were limited, and we had to cope with methodological difficulties to follow such small amounts in the body compartments. The sampling of urine is an especially critical step, as recovery of small amounts of fluids during 6 h is subjected to many experimental variations. We can also rise that the calculation of total body water is based upon body weight and not upon the precise determination of body composition. As the determination of body and urinary urea is the critical point in our assessment of protein quality, some uncertainties may have been introduced when using a rat model, even if each product was tested in the same conditions. Another interesting point in our results is that we reported a higher total oxidation for purified protein fractions (CAS and MSPI) than for other products (except SPRAY). MSPI was the most deaminated, this being consistent with studies performed in humans showing that dietary leucine was more markedly catabolized after the ingestion of whey than after that of casein (*39*). This higher deamination is due to the rapid delivery of dietary AAs from soluble milk protein (*48*).

Splanchnic deposition was measured in order to assess the proportion of dietary nitrogen available for the periphery. About 14−16.5% of dietary nitrogen was retained in the splanchnic organs and in plasma proteins 6 h after the meal, a finding that was very similar (14%) to that was already obtained in rats adapted to a normal protein diet (*17*). No differences were observed between the different groups except for SPRAY rats in which splanchnic sequestration reached 19%, because of a higher incorporation of dietary AAs in all splanchnic compartments (including the intestinal mucosa) as well as exported and constitutive liver proteins. Consequently, the nitrogen available for the periphery was the lowest in SPRAY rats reaching only 56% of dietary nitrogen while it was 62% in MSPI and between 64 and 67% in other groups. Insofar as peripheral AA disposal represents the amount of AAs available for the synthesis of peripheral proteins, especially muscle and skin, this could constitute a pertinent protein quality index. This index, in agreement with the other indexes measured during our study, i.e., digestibility, deamination, UPPN, and BV, revealed that as compared to the reference product (MF), SPRAY proteins had a significantly reduced protein quality. In addition, MSPI also exhibited lower BVs than other products while its protein digestibility-corrected AA score (PD-CAAS) reached 100% (*13*). This supports the idea that PD-CAAS is not a good reflection of protein quality measured in vivo (*49*). Indeed, the excellent PD-CAAS of MSPI is due to its AA composition and does not take into account the impact of protein kinetics on their nutritional quality.

In conclusion, this study combined the biochemical and structural characterization of heat-treated milk products and measurement of the postprandial utilization of dietary nitrogen in rats, using [^{15}N] methods. We found no correlation between the nutritional quality of milk proteins and the various heat treatments ranging from low pasteurization to UHT, despite the presence of a large quantity of heat treatment markers for UHT. This indicates that other markers beside those for heat treatment, such as lactosylation, are required to conclude as to loss of nutritional quality.

ABBREVIATIONS USED

α-Lac, α-lactalbumin; AA, amino acids; BCAA, branched chain amino acids; DAA, dispensable amino acids; IAA, indispensable amino acids; β-Lg, β-lactoglobulin; CAS, micellar casein(s); cfu, colony-forming unit; HHST, "higher temperature, shorter time" pasteurization; HTST, "high temperature, short time" pasteurization; LAL, lysinoalanine; MF, microfiltered milk; MSPI, milk soluble protein isolate; PAL, phosphatase alkaline; PD-CAAS, protein digestibility-corrected amino acid score; PER, protein efficiency ratio; RP-HPLC, reverse phase high-performance liquid chromatography; SPRAY, spray-dried milk (or spray drying); UHT, ultra heat-treated milk/product (or ultra heat treatment).

ACKNOWLEDGMENT

We are indebted to Arilait Recherches for their constructive scientific discussions. We are grateful to Catherine Luengo for her technical and skillful assistance all over the study. We thank M. Nicolas (AFSSA) for analyses of alkaline phosphatase and lactulose and N. Leconte, M. Piot, M. Goudedranche, and B. Camier-Caudron (UMR-STLO) for producing the different preparations of milks and milk proteins.

LITERATURE CITED

(1) Boschin, G.; D'Agostina, A.; Rinaldi, A.; Arnoldi, A. Lysinoalanine content of formulas for enteral nutrition. *J. Dairy Sci.* **2003**, *86* (7), 2283−2287.

(2) Henningfield, M. F.; Smith, S. D.; Reynolds, P. A.; Garcia, S. E.; Baxter, J. H. Protein quality of enteral nutrition products is consistent with label claims during shelf life and beyond expiration date. *J. Am. Diet. Assoc.* **1995**, *95* (1), 46−52.

(3) Estevez, A. M.; Castillo, E.; Figuerola, F.; Yanez, E. Effect of processing on some chemical and nutritional characteristics of pre-cooked and dehydrated legumes. *Plant Foods Hum. Nutr.* **1991**, *41* (3), 193−201.
(4) Friedman, M.; Brandon, D. L. Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* **2001**, *49* (3), 1069−1086.
(5) Ouedraogo, C. L.; Combe, E.; Lalles, J. P.; Toullec, R.; Treche, S.; Grongnet, J. F. Nutritional value of the proteins of soybeans roasted at a small-scale unit level in Africa as assessed using growing rats. *Reprod. Nutr. Dev.* **1999**, *39* (2), 201−212.
(6) Friedman, M. Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *J. Agric. Food Chem.* **1999**, *47* (4), 1295−1319.
(7) Bertrand-Harb, C.; Baday, A.; Dalgalarrondo, M.; Chobert, J. M.; Haertle, T. Thermal modifications of structure and co-denaturation of alpha-lactalbumin and beta-lactoglobulin induce changes of solubility and susceptibility to proteases. *Nahrung* **2002**, *46* (4), 283−289.
(8) Friedman, M.; Gumbmann, M. R.; Masters, P. M. Protein-alkali reactions: Chemistry, toxicology, and nutritional consequences. *Adv. Exp. Med. Biol.* **1984**, *177*, 367−412.
(9) Wenzel, E.; Tasto, S.; Erbersdobler, H. F.; Faist, V. Effect of heat-treated proteins on selected parameters of the biotransformation system in the rat. *Ann. Nutr. Metab.* **2002**, *46* (1), 9−16.
(10) Dalgleish, D. G. Denaturation and aggregation of serum proteins and caseins in heated milk. *J. Agric. Food Chem.* **1990**, *38* (11), 1995−1999.
(11) al Kanhal, H. A.; al-Othman, A. A.; Hewedi, F. M. Changes in protein nutritional quality in fresh and recombined ultrahigh-temperature treated milk during storage. *Int. J. Food Sci. Nutr.* **2001**, *52* (6), 509−514.
(12) El, S. N.; Kavas, A. Available lysine in dried milk after processing. *Int. J. Food Sci. Nutr.* **1997**, *48* (2), 109−111.
(13) Gilani, G. S.; Sepehr, E. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. *J. Nutr.* **2003**, *133* (1), 220−225.
(14) D'Agostina, A.; Boschin, G.; Rinaldi, A.; Arnoldi, A. Updating on the lysinoalanine content of commercial infant formulae and beicost products. *Food Chem.* **2003**, *80*, 483−488.
(15) Rutherfurd, S. M.; Moughan, P. J. Application of a new method for determining digestible reactive lysine to variably heated protein sources. *J. Agric. Food Chem.* **1997**, *45* (5), 1582−1586.
(16) Morens, C.; Gaudichon, C.; Fromentin, G.; Marsset-Baglieri, A.; Bensaid, A.; Larue-Achagiotis, C.; Luengo, C.; Tome, D. Daily delivery of dietary nitrogen to the periphery is stable in rats adapted to increased protein intake. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *281* (4), E826−E836.
(17) Morens, C.; Gaudichon, C.; Metges, C. C.; Fromentin, G.; Baglieri, A.; Even, P. C.; Huneau, J. F.; Tome, D. A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. *J. Nutr.* **2000**, *130* (9), 2312−2321.
(18) Bos, C.; Mahe, S.; Gaudichon, C.; Benamouzig, R.; Gausseres, N.; Luengo, C.; Ferriere, F.; Rautureau, J.; Tome, D. Assessment of net postprandial protein utilization of 15N-labeled milk nitrogen in human subjects. *Br. J. Nutr.* **1999**, *81* (3), 221−226.
(19) Bos, C.; Metges, C. C.; Gaudichon, C.; Petzke, K. J.; Pueyo, M. E.; Morens, C.; Everwand, J.; Benamouzig, R.; Tome, D. Postprandial kinetics of dietary amino acids are the main determinant of their metabolism after soy or milk protein ingestion in humans. *J. Nutr.* **2003**, *133* (5), 1308−1315.
(20) Reeves, P. G. Components of the AIN-93 diets as improvements in the AIN-76A diet. *J. Nutr.* **1997**, *127* (5 Suppl.), 838S−841S.
(21) FIL-IDF Standard 147B. Heat treated milk—Determination of lactulose content, 1998.
(22) FIL-IDF Standard 155-2: 2003/ISO 11816-2. Milk and milk products—Determination of alkaline phosphatase activity, 2003.
(23) Spackman, D. H.; Stein, W. H.; Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **1958**, *30*, 1190−1206.
(24) Leonil, J.; Molle, D.; Fauquant, J.; Maubois, J. L.; Pearce, R. J.; Bouhallab, S. Characterization by ionization mass spectrometry of lactosyl beta-lactoglobulin conjugates formed during heat treatment of milk and whey and identification of one lactose-binding site. *J. Dairy Sci.* **1997**, *80* (10), 2270−2281.
(25) Gaudichon, C.; Mahe, S.; Benamouzig, R.; Luengo, C.; Fouillet, H.; Dare, S.; Van Oycke, M.; Ferriere, F.; Rautureau, J.; Tome, D. Net postprandial utilization of [15N]-labeled milk protein nitrogen is influenced by diet composition in humans. *J. Nutr.* **1999**, *129* (4), 890−895.
(26) Gausseres, N.; Mahe, S.; Benamouzig, R.; Luengo, C.; Ferriere, F.; Rautureau, J.; Tome, D. [15N]-labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. *J. Nutr.* **1997**, *127* (6), 1160−1165.
(27) Sharp, P. E.; La Regina, M. C. *The Laboratory Rat*; CRC Press LLC: Boca Raton, FL, 1998.
(28) Waynforth, H. B.; Flecknell, P. A. *Experimental and Surgical Technique in the Rat*; Academic Press: New York, 1998.
(29) Desrosiers, T.; Savoie, L.; Bergeron, G.; Parent, G. Estimation of lysine damage in heated whey proteins by furosine determinations in conjonction with the digestion cell technique. *J. Agric. Food Chem.* **1989**, *37* (5), 1385−1391.
(30) Donoso, G.; Lewis, O. A. M.; Miller, D. S.; Payne, P. R. Effect of heat treatment on the nutritive value of proteins: Chemical and balance studies. *J. Sci. Food Agric.* **1962**, *13*, 192−197.
(31) Hurrell, R. F.; Finot, P. A. Food processing and storage as a determinant of protein and amino acid availability. *Exp. Suppl.* **1983**, *44*, 135−156.
(32) Rerat, A.; Calmes, R.; Vaissade, P.; Finot, P. A. Nutritional and metabolic consequences of the early Maillard reaction of heat treated milk in the pig. Significance for man. *Eur. J. Nutr.* **2002**, *41* (1), 1−11.
(33) Bouhallab, S.; Morgan, F.; Henry, G.; Molle, D.; Leonil, J. Formation of stable covalent dimer explains the high solubility at pH 4.6 of lactose-β-lactoglobulin conjugates heated near neutral pH. *J. Agric. Food Chem.* **1999**, *47* (4), 1489−1494.
(34) de Vrese, M.; Frik, R.; Roos, N.; Hagemeister, H. Protein-bound D-amino acids, and to a lesser extent lysinoalanine, decrease true ileal protein digestibility in minipigs as determined with (15)N-labeling. *J. Nutr.* **2000**, *130* (8), 2026−2031.
(35) Murry, A. C., Jr.; Gelaye, S.; Casey, J. M.; Foutz, T. L.; Kouakou, B.; Arora, D. Type of milk consumed can influence plasma concentrations of fatty acids and minerals and body composition in infant and weanling pigs. *J. Nutr.* **1999**, *129* (1), 132−138.
(36) Rudloff, S.; Lonnerdal, B. Solubility and digestibility of milk proteins in infant formulas exposed to different heat treatments. *J. Pediatr. Gastroenterol. Nutr.* **1992**, *15* (1), 25−33.
(37) Sarwar, G.; Peace, R. W.; Botting, H. G. Differences in protein digestibility and quality of liquid concentrate and powder forms of milk-based infant formulas fed to rats. *Am. J. Clin. Nutr.* **1989**, *49* (5), 806−813.
(38) Rutherfurd, S. M.; Moughan, P. J. The rat as a model animal for the growing pig in determining ileal amino acid digestibility in soya and milk proteins. *J. Anim. Physiol. Anim. Nutr. (Berlin)* **2003**, *87* (7−8), 292−300.
(39) Boirie, Y.; Dangin, M.; Gachon, P.; Vasson, M. P.; Maubois, J. L.; Beaufrere, B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94* (26), 14930−14935.
(40) Daniel, H.; Vohwinkel, M.; Rehner, G. Effect of casein and β-casomorphins on gastrointestinal motility in rats. *J. Nutr.* **1990**, *120* (3), 252−257.
(41) Mahe, S.; Roos, N.; Benamouzig, R.; Davin, L.; Luengo, C.; Gagnon, L.; Gausserges, N.; Rautureau, J.; Tome, D. Gastrojejunal kinetics and the digestion of [^{15}N]β-lactoglobulin and casein in humans: The influence of the nature and quantity of the protein. *Am. J. Clin. Nutr.* **1996**, *63* (4), 546−552.

(42) Evangelisti, F.; Calcagno, C.; Nardi, S.; Zunin, P. Deterioration of protein fraction by Maillard reaction in dietetic milks. *J. Dairy Res.* **1999**, *66* (2), 237−243.
(43) Bos, C.; Gaudichon, C.; Tome, D. Isotopic studies of protein and amino acid requirements. *Curr. Opin. Clin. Nutr. Metab. Care* **2002**, *5* (1), 55−61.
(44) Morens, C.; Bos, C.; Pueyo, M. E.; Benamouzig, R.; Gausseres, N.; Luengo, C.; Tome, D.; Gaudichon, C. Increasing habitual protein intake accentuates differences in postprandial dietary nitrogen utilization between protein sources in humans. *J. Nutr.* **2003**, *133* (9), 2733−2740.
(45) Millward, D. J.; Fereday, A.; Gibson, N. R.; Pacy, P. J. Human adult amino acid requirements: [1−13C]Leucine balance evaluation of the efficiency of utilization and apparent requirements for wheat protein and lysine compared with those for milk protein in healthy adults. *Am. J. Clin. Nutr.* **2000**, *72* (1), 112−121.
(46) Guyomarc'h, F.; Law, A. J.; Dalgleish, D. G. Formation of soluble and micelle-bound protein aggregates in heated milk. *J. Agric. Food Chem.* **2003**, *51* (16), 4652−4660.
(47) Smits, P.; Van Brouwershaven, J. H. Heat-induced association of β-lactoglobulin and casein micelles. *J. Dairy Res.* **1980**, *47* (3), 313−325.
(48) Dangin, M.; Boirie, Y.; Garcia-Rodenas, C.; Gachon, P.; Fauquant, J.; Callier, P.; Ballevre, O.; Beaufrere, B. The digestion rate of protein is an independent regulating factor of postprandial protein retention. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280* (2), E340−E348.
(49) Schaafsma, G. The protein digestibility-corrected amino acid score. *J. Nutr.* **2000**, *130* (7), 1865S−1867S.

Received for review June 2, 2005. Revised manuscript received September 26, 2005. Accepted October 11, 2005. This work was supported by Arilait Recherches and the French Office of Research and Technology.

JF051304D