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Available lysine and fluorescence in heated milk proteins/dextrinomaltose or lactose solutions

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Abstract

In order to predict and compare the effects of dextrinomaltose and lactose on available lysine loss by the Maillard reaction, six model systems were prepared by mixing casein, laboratory whey protein or commercial whey protein with dextrinomaltose or lactose. The solutions were prepared at concentrations similar to those used in enteral and infant formula processing and were heated at 100, 120 or 140 °C for 0–30 min. The progress of the Maillard reaction in these model systems was followed by monitoring free fluorescence intermediary compounds. Model systems with lactose showed higher available lysine less than the model systems with dextrinomaltose; linear lysine losses were obtained between 0 and 20 min at 100 and 120 °C. At sterilization temperature and time (120 °C/10 min), lysine losses of milk proteins with dextrinomaltose as reducing sugar were 6.1% for casein, 4.1% for laboratory whey protein and 13.4% for commercial whey protein. Available lysine showed correlation with furosine in model systems prepared with lactose and casein or laboratory but not commercial whey protein at 100 and 120 °C. The initial fluorescence value obtained by mixing casein or laboratory whey protein with lactose or dextrinomaltose was low (between 3.8 and 5.7), whereas the value obtained when commercial whey proteins were used was close to 9. At 120 °C/10 min, there was only a small increase of fluorescence in casein and laboratory whey protein but a large increase in commercial whey protein (threefold the initial value). Fluorescence measurement is useful for finding the extent of the Maillard reaction in commercial whey protein (thermally damaged protein). An absolute value greater than 10 may indicate that products were prepared with thermally damaged proteins.

Keywords: Available lysine; Fluorescence; Milk proteins; Dextrinomaltose; Lactose

1. Introduction

Enteral formulas are the products most commonly used to feed patients who are unable or unwilling to eat normally (Rombeau, 1997). They are prepared by mixing proteins, carbohydrates, fats and other nutrients (Mora, 1997). These ingredients can be affected by the heat treatment used in their sterilization and by the environmental conditions (e.g. temperature and light exposure) of their storage.

Non-enzymatic browning, known as the Maillard reaction (MR), occurs in many foods, including dairy products. In an early step, the free amino groups of the proteins react non-enzymatically with reducing sugars. In the advanced step of the reaction, proteins are modified into coloured, fluorescent and cross-linked molecules. The MR plays an important role in the production of undesirable organoleptic characteristics (flavour and colour) (Ferretti & Flanagan, 1972) and in the decrease of nutritional quality, especially loss of lysine (Baltes, 1982).

Various chemical methods have been developed to measure nutritionally available lysine, considered as lysine units that have not combined with other food

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ingredients and still have free reactive ε-amino groups (Hurrell & Carpenter, 1981). They employ several reagents with variable specificity for the ε-amino group of lysine. The most widespread method, developed by Carpenter (1960), uses 1-fluoro-2,4-dinitrobenzene (FDNB). Chromatographic techniques have been applied to separate ε-DNP-lysine from interfering aminoacids and other compounds (Rabasseda, Rauret, & Galceran, 1988).

There have been several studies of lysine loss due to heat treatment and storage of infant formula (Anantanaman & Finot, 1993; Castillo, Sanz, Serrano, & Hernández, 2002; Guerra-Hernández, Leon, García-Villanova, Corzo, & Romera, 2002a, Guerra-Hernández, Leon, García-Villanova, Corzo, & Romera, 2002b; Rossi & Pompei, 1991) and model systems (Baisier & Labuza, 1992; Morales, Romero, & Jiménez-Pérez, 1996).

Fluorescent compounds are formed in stages before the formation of brown pigments, as described in detail by Baisier and Labuza (1992), Burton, McWeeny, and Biltcliffe (1963) Morales, Romero, and Jiménez Pérez (1995) in model systems of amines/sugars and milk. Baisier and Labuza (1992) reported that fluorescence accumulation in a glucose/lysine system was due to irreversible interaction between reactive reducing compounds and amines. The formation of most fluorescent compounds is linked to the presence of casein and occurs (simultaneously) with browning (Tarassuk & Simonson, 1950). Morales et al. (1996) found a linear correlation between the time and temperature of heat treatment and the fluorescence in milk and in model systems. Fluorescence was also shown to be useful for determining the extent of the browning reaction during the manufacture and storage of enteral formulas (Rufián-Henares, García-Villanova, & Guerra-Hernández, 2002a, 2002b).

The purpose of this study was to determine the behaviour of available lysine and fluorescence during the processing of model systems prepared with ingredients similar to those used in enteral and infant formula manufacture. The ingredients present varying degrees of thermal damage.

2. Materials and methods

2.1. Samples

All chemicals used were of analytical grade, from Sigma–Aldrich (Madrid, Spain). A Spanish dietetic product company provided the components calcium caseinate and whey proteins (WPI). Lactose-1-hydrate (analytical grade) was from Panreac (Barcelona, Spain) and dextrinomaltose (95% carbohydrate, DE = 19) was from Novartis (Barcelona, Spain). Proteins with low heat damage (called "laboratory whey proteins") were obtained in our laboratory from raw milk. Briefly, 5 l of raw milk were skimmed by centrifugation at 4000 rpm (14,000*g*) for 10 min at 4 °C. Then, casein was precipitated by acidification with 6 N HCl to pH 4.6 and centrifugation. The whey obtained was frozen at −80 °C and lyophilized. In order to remove lactose, the lyophilized whey was re-dissolved in 500 ml of deionized water with 2% thymol as preservative and dialyzed at 4 °C for 10 days, using a size exclusion membrane of 8000 Daltons. The water was changed on alternate days. Finally, the delactosed whey was again lyophilized.

Model systems were made by dissolving calcium caseinate, commercial whey proteins or laboratory whey proteins (4% w/v) with lactose (7% w/v) or dextrinomaltose (12% w/v) in 100 ml of 0.1 M phosphate buffer at pH 6.5. Two aliquots of 10 ml were then placed in Pyrex screw-cap vials, which were immersed for 5–30 min in a glycerol bath kept at 100, 120 and 140 °C. The samples were then cooled in an ice bath and stored at −50 °C prior to analysis. The heating times reported exclude the heating-up period, which was estimated at 2 min.

2.2. Methods

2.2.1. Available lysine

ε-NDP-lysine was determined by HPLC, following the method applied to infant cereals by Fernández-Artigas, García-Villanova, and Guerra-Hernández (1999) with some modifications (Ramirez-Jimenez, García-Villanova, & Guerra-Hernández, 2004). The model system was diluted 1/10 and 1 ml of this dilution was then placed in the bottom of a 25 ml Pyrex screw-cap vessel tube with PTFE-faced septa, and 1 ml $NaHCO_3$ (8%) solution and 1.5 ml FDNB (3% FDNB in ethanol) solution were added. The closed tubes were mechanically shaken for 3 h at room temperature and ethanol was evaporated by immersing them in a 95 °C water bath. The hydrolysis of FDNB derivate solutions was performed with 3 ml of 8.1 M HCl in an oven at 110 °C for 24 h, after the removal of CO_2 by stirring. The hydrolyzed solution was filtered and pH 5 was reached with 6 M NaOH and 1 M $NaHCO_3$; the volume was adjusted with methanol: 0.01 M sodium acetate, pH 5, buffer (1:1) solution to 25 ml, and 3 ml of this solution were cleaned with diethyl ether (three times), removing the ether with, Pasteur pipette and nitrogen. The solution was filtered through 0.2 μm disc filter.

The liquid chromatography study was performed in a Perkin–Elmer 250 model with a Waters 717 automatic injector and Perkin–Elmer 235 UV diode array detector. The integrator-computer used was a 1020 model Perkin–Elmer Nelson. Fifty microlitres of filtered solution were separated in a reverse-phase C_{18} HPLC column (Nova-Pak C_{18}, 250 × 3.9 mm id; Waters) operating at room temperature. The mobile phase was methanol: 0.01 M sodium acetate, pH 5, buffer (1:1). The elution was isocratic and the flow rate was 1 ml/min. The UV detector

was set at 360 nm. The run time was 15 min and ε-DNP-lysine was completely separated in 6 min. The determination of ε-DNP-lysine was carried out by the external standard method.

2.2.2. Maillard fluorescent compounds

Samples were prepared by the method of Morales et al. (1996). Five millilitres of well mixed sample were deproteinized with 5 ml of trichloroacetic acid solution (40% w/v) and centrifuged for 10 min at 4500 rpm (Heraeus Christ GMBH). Then, the supernatant was filtered through a Whatman 42 filter paper and 500 μl of sample were diluted with 2.5 ml of phosphate buffered saline (20 mM, pH 7.0 and 15 mM NaCl). The measurements were performed on a model 1501 Shimadzu fluorescence spectrophotometer at 345 nm excitation and 415 emission. A quinine sulphate solution of 0.1 μg/ml in 0.1 N H_2SO_4 was daily prepared as standard for calibration of the instrument at 100% relative fluorescence.

2.3. Statistical analysis

Statistical analysis of data was performed by analysis of variance (Microcal Origin 5.0, Microcal Software, Northampton, MA). The Student's *t*-test was used to compare means and the level of significance was set at 99.9%.

3. Results and discussion

3.1. Available lysine

3.1.1. Preliminary studies

The samples showed an interference peak at $R_T = 2.3$ min that impeded the correct quantification of DNP-lysine. In order to obtain a better quantification, a previous treatment with diethyl ether was necessary to remove most of the interference peak (Fig. 1).

Precision was assayed in a casein-dextrinomaltose model system, giving a relative standard deviation of 2.21% for seven samples and an average value of 6295 mg/100 g of protein. The precision obtained allows the evaluation of available lysine variations during the thermal treatment of model systems.

3.1.2. Sample analysis

Available lysine values are shown in Tables 1, 2, 3, 4, 5, 6. In all systems, losses were higher when time and heating temperature were increased. Samples with lactose showed higher losses than did samples with dextrinomaltose. Model systems with whey proteins showed higher losses versus systems with casein because of the higher lysine contents of the former. At 100 °C, the losses were greater in systems with commercial than with laboratory whey proteins, whereas an inverse tendency

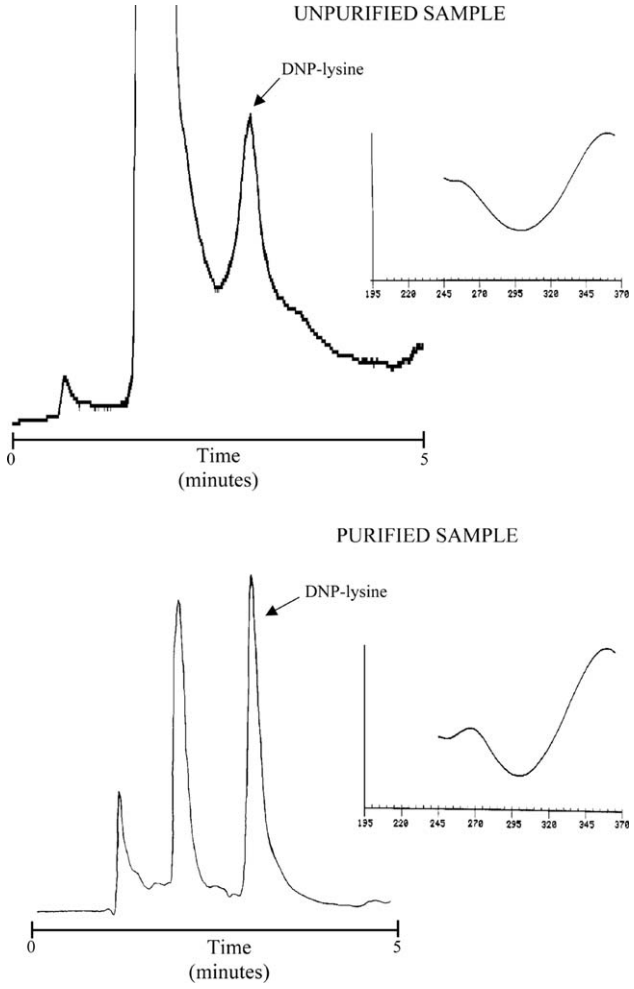

Fig. 1. Chromatograms and spectra of purified and unpurified samples.

was observed at 140 °C. The denaturation of whey proteins, which may favour the Maillard reaction, is greater in the commercial product at relatively low temperatures (100 °C) and in the laboratory product at higher temperatures. This fact, along with the greater lysine content of laboratory versus commercial whey proteins, may explain the above finding.

The casein–lactose model system showed statistically significant losses ($P < 0.001$) of 7.3–49.0% after 30 min of heating at 100–140 °C. In the casein-dextrinomaltose model system, losses ranged from 4.8% to 38.5% for the same temperature and duration of treatment ($P < 0.001$). After 10 min at 120 °C (the usual time and temperature for sterilising products prepared with these ingredients), the losses were 9.6% and 6.1% in the systems with lactose and dextrinomaltose, respectively. These losses are lower than the 16–33% losses reported by Castillo et al. (2002) in their analysis of commercial enteral formulae with casein as protein source, although the processing and the carbohydrate content of the formulae with casein were not reported. Brands and Van

Table 1
Available lysine content in casein–lactose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	7907 ± 86	–	7907 ± 86	–	7907 ± 86	–
5	7870 ± 71	0.46	7479 ± 64	4.98	6983 ± 74	11.7
10	7813 ± 60	1.18	7146 ± 43	9.63	6370 ± 38	19.4
15	7732 ± 44	2.11	6694 ± 53	15.3	5785 ± 23	26.8
20	7654 ± 103	3.20	6400 ± 75	19.1	5148 ± 130	38.9
25	7544 ± 107	4.54	6080 ± 83	23.1	4641 ± 63	41.3
30	7331 ± 38	7.28	5758 ± 38	27.2	4026 ± 130	49.1

$n = 2$.

Table 2
Available lysine content in casein-dextrinomaltose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	7926 ± 43	–	7926 ± 43	–	7926 ± 43	–
5	7908 ± 44	0.2	7625 ± 31	3.5	7210 ± 99	9.0
10	7857 ± 39	0.8	7439 ± 109	6.1	6705 ± 102	15.4
15	7805 ± 84	1.5	7168 ± 78	9.5	6215 ± 69	21.5
20	7761 ± 35	2.0	6932 ± 91	12.5	5709 ± 73	27.9
25	7684 ± 106	3.0	6688 ± 94	15.6	5273 ± 108	33.4
30	7543 ± 131	4.8	6434 ± 95	18.8	4872 ± 48	38.5

$n = 2$.

Table 3
Available lysine content in laboratory whey proteins–lactose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	8712 ± 4	–	8712 ± 4	–	8712 ± 5	–
5	8811 ± 86	−1.14	8248 ± 104	5.32	7517 ± 80	13.7
10	8780 ± 95	−0.79	7721 ± 31	11.4	6510 ± 60	25.3
15	8660 ± 74	0.60	7146 ± 53	18.0	5352 ± 98	38.6
20	8528 ± 121	2.11	6578 ± 73	24.5	4349 ± 118	50.1
25	8191 ± 90	5.98	6110 ± 77	29.9	3406 ± 94	60.9
30	7836 ± 108	10.1	5375 ± 44	38.3	2360 ± 51	72.9

$n = 2$.

Table 4
Available lysine content in laboratory whey proteins-dextrinomaltose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	8791 ± 54	–	8791 ± 54	–	8791 ± 54	–
5	8775 ± 88	0.17	8688 ± 50	1.00	8388 ± 170	4.58
10	8746 ± 127	0.51	8429 ± 101	4.11	7486 ± 75	14.9
15	8728 ± 145	0.71	8147 ± 84	7.33	6625 ± 109	24.6
20	8671 ± 75	1.36	7826 ± 27	11.0	5801 ± 56	34.0
25	8493 ± 87	3.39	7597 ± 73	13.6	5011 ± 22	43.0
30	8231 ± 135	6.37	6740 ± 15	23.3	4098 ± 121	53.4

$n = 2$.

Boekel (2001) found lysine losses of 47% in casein and glucose model systems (3% casein and 2.7% glucose) heated at 120 °C for 30 min.

Model systems with whey proteins and lactose heated at 100–140 °C for 30 min showed statistically significant ($P < 0.001$) losses (10.0–73.0% in the system with labo-

Table 5
Available lysine content in commercial whey proteins-lacose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	7446 ± 32	–	7446 ± 32	–	7446 ± 32	–
5	6987 ± 70	6.17	6769 ± 48	3.12	6533 ± 22	12.3
10	6808 ± 46	8.58	6349 ± 56	14.7	5805 ± 57	22.1
15	6621 ± 172	11.1	5979 ± 90	19.7	5201 ± 67	30.2
20	6404 ± 49	14.0	5465 ± 90	26.6	4498 ± 37	39.6
25	6069 ± 69	18.5	5012 ± 35	32.7	3774 ± 21	49.3
30	5615 ± 35	24.6	4527 ± 89	39.2	3194 ± 103	57.1

$n = 2$.

Table 6
Available lysine content in commercial whey proteins-dextrinomaltose model system

Time (min)	100 °C		120 °C		140 °C	
	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)	mg/100 g protein	Loss (%)
0	7469 ± 94	–	7469 ± 94	–	7469 ± 94	–
5	7039 ± 81	5.76	6795 ± 45	3.46	6631 ± 23	11.2
10	6909 ± 121	7.50	6466 ± 67	13.4	6005 ± 47	19.6
15	6742 ± 89	9.75	6093 ± 120	18.4	5461 ± 67	26.9
20	6575 ± 27	12.0	5729 ± 198	23.3	4865 ± 113	34.9
25	6372 ± 92	14.7	5118 ± 102	31.5	4372 ± 116	41.5
30	6172 ± 60	17.4	4848 ± 129	35.1	3768 ± 70	49.6

$n = 2$.

ratory whey proteins and 25.0–57.0% in system with commercial whey proteins), which were greater than those observed in the casein–lactose model system after the same treatment. A similar finding was observed in the model systems with dextrinomaltose. After sterilization at 120 °C for 10 min, the losses were 11.4% in the laboratory whey protein–lactose model system and 14.7% in the commercial whey protein–lactose system. Losses in similar model systems prepared with dextrinomaltose were 4.1% in those with laboratory whey proteins and 13.4% in those with commercial whey proteins.

If proteins with low thermal damage (casein and laboratory whey proteins) are used in the preparation of formula, sterilization temperatures can produce nutritional losses. These losses, determined as available lysine, can be around 5% when the carbohydrate is dextrinomaltose and 10% when it is lactose. Moreover, the residual lysine level will be higher in non-thermally damaged proteins.

Table 7 shows the data obtained after 20 min of thermal treatment at 100 and 120 °C. Lysine losses were linear under these conditions, allowing the use of this parameter to predict nutritional losses of protein ingredients after sterilisation.

Finot, Deutsh, and Bujard (1981) established a correlation between furosine and blocked lysine in milk, and this correlation was later applied in infant formulas (Evangelisti, Calcagno, & Zunin, 1994). For this relationship to be applied in enteral formulae, a correlation between furosine generation and lysine loss must be demonstrated. Table 8 shows the linear correlation coefficients between furosine (Rufian-Henares, 2002) and available lysine in different model systems. There was no correlation in model systems prepared with commercial whey proteins at any of the temperatures assayed and, in the other model systems there was no correlation at 140 °C. Model systems containing lactose and non-thermally damaged proteins (casein and laboratory

Table 7
Available lysine time–temperature correlation

Model system	100 °C	120 °C
Casein–lactose	$y = -12.9x + 7924$ ($r^2 = 0.9787$)	$y = -75.9x + 7884$ ($r^2 = 0.9963$)
Casein-dextrinomaltose	$y = -8.6x + 7937$ ($r^2 = 0.9794$)	$y = -48.9x + 7907$ ($r^2 = 0.9963$)
Laboratory whey proteins–lactose	$y = -13.5x + 8849$ ($r^2 = 0.8001$)	$y = -110.6x + 8802$ ($r^2 = 0.9997$)
Laboratory whey proteins-dextrinomaltose	$y = -5.7x + 8799$ ($r^2 = 0.9401$)	$y = -49.4x + 8870$ ($r^2 = 0.9732$)
Commercial whey proteins–lactose	$y = -49.0x + 7343$ ($r^2 = 0.9573$)	$y = -95.1x + 7352$ ($r^2 = 0.9885$)
Commercial whey proteins-dextrinomaltose	$y = -41.7x + 7364$ ($r^2 = 0.9393$)	$y = -83.7x + 7347$ ($r^2 = 0.9789$)

y = Available lysine content (mg/100 g of protein); x = time (min).

Table 8
Furosine-available lysine correlation (r^2) in model systems

Model system	100 °C	120 °C	140 °C
Casein–lactose	−0.9139	−0.9986	−0.2284
Casein-dextrinomaltose	−0.9426	−0.8985	−0.5695
Laboratory whey proteins–lactose	−0.9319	−0.9829	0.8987
Laboratory whey proteins-dextrinomaltose	−0.8660	−0.6530	0.9519
Commercial whey proteins–lactose	0.9630	0.9155	0.9586
Commercial whey proteins-dextrinomaltose	0.9942	0.8910	0.9209

whey proteins) showed a linear correlation of −0.92 at 100 °C and −0.99 at 120 °C. In model systems with dextrinomaltose, this correlation was −0.90 for casein and lower in model systems with laboratory whey proteins, especially at 120 °C ($r = -0.6525$).

These results indicate that the formula established by Finot et al. (1981) may be applied to products with non-thermally damaged ingredients, such as milk. However, furosine measurement is unable to show nutritional losses during the manufacture of enteral formulae prepared with damaged proteins. It can only be applied in formulae that have casein as the sole source of protein.

3.2. Fluorescence

3.2.1. Preliminary studies

Fluorimetric measurement was performed using the supernatant obtained after protein precipitation with trichloroacetic acid (Morales et al., 1996). The RSD obtained was 1.72% for nine samples.

3.2.2. Sample analysis

Tables 9–11, show the results of the fluorescence analysis in model systems with casein, laboratory whey proteins or commercial whey proteins and dextrinomaltose or lactose. The changes were statistically significant ($P < 0.001$) at 140 °C for all the times assayed in all model systems. At 100 and 120 °C, model systems with casein and laboratory whey proteins showed significant changes after every 10 min of heat treatment whereas, in model systems with commercial whey proteins, the changes were significant at all the times assayed (every 5 min).

The reactivities were very similar between model systems prepared with casein or laboratory whey proteins. Systems with lactose showed a slightly higher reactivity than did systems with dextrinomaltose.

The results for commercial whey proteins were very different, and major changes were observed, even at 100 °C. The values obtained in casein and laboratory whey proteins after 30 min, at temperatures ⩽120 °C,

Table 9
Relative fluorescence intensity (FI%) in casein model systems

Time (min)	Lactose			Dextrinomaltose		
	100 °C	120 °C	140 °C	100 °C	120 °C	140 °C
0	5.7 ± 0.0	5.7 ± 0.0	5.7 ± 0.0	5.2 ± 0.0	5.2 ± 0.0	5.2 ± 0.0
5	5.8 ± 0.0	6.1 ± 0.1	16.8 ± 0.1	5.2 ± 0.1	5.5 ± 0.2	14.7 ± 0.2
10	5.9 ± 0.1	6.6 ± 0.0	26.8 ± 0.1	5.3 ± 0.1	5.8 ± 0.1	23.4 ± 0.3
15	6.1 ± 0.1	7.1 ± 0.1	38.5 ± 0.7	5.4 ± 0.2	6.2 ± 0.1	33.0 ± 0.2
20	6.3 ± 0.1	7.7 ± 0.1	49.0 ± 0.3	5.6 ± 0.1	6.6 ± 0.1	42.1 ± 0.5
25	6.5 ± 0.1	8.1 ± 0.0	60.2 ± 0.2	5.6 ± 0.1	6.9 ± 0.1	51.1 ± 0.1
30	6.7 ± 0.2	8.7 ± 0.1	70.9 ± 0.4	5.7 ± 0.1	7.2 ± 0.2	60.4 ± 0.2

$n = 4$.

Table 10
Relative fluorescence intensity (FI%) in laboratory whey protein model system

Time (min)	Lactose			Dextrinomaltose		
	100 °C	120 °C	140 °C	100 °C	120 °C	140 °C
0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.0
5	4.9 ± 0.0	5.2 ± 0.1	11.0 ± 0.4	4.3 ± 0.0	4.9 ± 0.1	9.1 ± 0.0
10	5.5 ± 0.1	6.1 ± 0.4	20.5 ± 0.4	4.6 ± 0.1	5.6 ± 0.0	16.0 ± 0.1
15	6.0 ± 0.2	7.0 ± 0.2	30.8 ± 0.8	5.1 ± 0.1	6.5 ± 0.0	25.1 ± 0.1
20	6.6 ± 0.2	8.0 ± 0.4	42.5 ± 0.8	5.5 ± 0.1	7.2 ± 0.1	34.1 ± 0.7
25	7.1 ± 0.2	9.0 ± 0.1	53.6 ± 0.5	5.8 ± 0.1	7.9 ± 0.1	42.5 ± 0.4
30	7.7 ± 0.3	10.0 ± 0.2	64.8 ± 0.3	6.1 ± 0.1	8.6 ± 0.1	50.8 ± 0.2

$n = 4$.

Table 11
Relative fluorescence intensity (FI%) in commercial whey protein model systems

Time (min)	Lactose			Dextrinomaltose		
	100 °C	120 °C	140 °C	100 °C	120 °C	140 °C
0	9.3 ± 0.0	9.3 ± 0.0	9.3 ± 0.0	8.6 ± 0.0	8.6 ± 0.0	8.6 ± 0.0
5	9.7 ± 0.4	17.2 ± 0.1	25.0 ± 0.2	9.7 ± 0.2	16.6 ± 0.4	21.5 ± 0.4
10	12.1 ± 0.1	25.0 ± 0.8	42.8 ± 0.5	11.0 ± 0.4	23.5 ± 0.8	34.2 ± 0.2
15	14.2 ± 0.2	30.5 ± 0.2	65.3 ± 0.3	12.6 ± 0.3	28.7 ± 0.4	48.7 ± 0.1
20	17.6 ± 0.7	36.2 ± 0.3	86.5 ± 0.4	14.9 ± 0.6	32.9 ± 0.5	65.2 ± 0.2
25	20.0 ± 0.6	41.7 ± 0.5	104 ± 0.9	16.9 ± 0.1	37.4 ± 0.6	79.6 ± 0.7
30	23.6 ± 0.2	48.3 ± 0.2	122 ± 0.8	19.4 ± 0.1	43.0 ± 0.3	93.0 ± 1.0

$n = 4$.

were lower than 10 units. These ingredients have low thermal damage, measured as furosine determination (Rufian-Henares, 2002). For commercial whey proteins, the values obtained after 10 min of heating (a sterilisation procedure when 120 °C) were fourfold higher. These proteins have high thermal damage, measured by pyrraline determination (Rufian-Henares, Guerra-Hernandez, & García-Villanova, 2004).

Morales and Van Boeckel (1997) studied lactose-casein and lactose-whey protein systems at 90, 120, 130 and 140 °C and found significant changes at temperatures $\geqslant$120 °C. Rufián-Henares et al. (2002a) determined fluorescence intensity during enteral formula manufacture and reported an increase in this indicator in heat-treated samples (UHT and sterilised) as a result of an increase in the browning. Sterilisation produced higher fluorescence intensity values than did UHT because of its longer heating time (9 min/120 °C versus 4 s/132 °C). Another study (Rufián-Henares, García-Villanova, & Guerra-Hernández, 2002b) of enteral formula storage and fluorescence showed an increase in free fluorescent intermediate compounds in enteral formulae with longer times (12, 24 and 36 weeks) and higher temperatures (4, 20, 30 and 55 °C) of storage.

Pyrraline is a useful indicator to control thermally damaged ingredients (Rufian-Henares et al., 2004). The correlation between fluorescence and pyrraline was studied in commercial whey protein and lactose or dextrinomaltose model systems heated at 120 °C. The linear correlation obtained was $r = 0.9486$ for the model system with lactose and $r = 0.9678$ for that with dextrinomaltose. When only the first 20 min were considered (i.e., including any type of sterilisation), the correlation was even stronger, at 0.9933 and 0.9997 for model systems with lactose and dextrinomaltose, respectively. Fluorescence measurement is simpler and cheaper than pyrraline determination.

In conclusion, available lysine values are much lower in thermally damaged ingredients than in non-damaged ones. Under sterilisation conditions, nutritional losses are greater in systems prepared with damaged ingredients than in those with undamaged ingredients. For the assessment of nutritional quality, furosine determination is useful in systems prepared with ingredients with low thermal damage, whereas fluorescence measurement is useful in systems prepared with thermally damaged ingredients. A value of more than 10 units of fluorescence may indicate that a product was prepared with thermally damaged ingredients.

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