

Heat-induced and other chemical changes in commercial UHT milks

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The properties of commercial directly and indirectly heated UHT milks, both after heating and during storage at room temperature for 24 weeks, were studied. Thermally induced changes were examined by changes in lactulose, furosine and acid-soluble whey proteins. The results confirmed previous reports that directly heated UHT milks suffer less heat damage than indirectly heated milk. During storage, furosine increased and bovine serum albumin in directly heat-treated milks decreased significantly. The changes in lactulose, α -lactalbumin and β -lactoglobulin were not statistically significant. The data suggest that heat treatment indicators should be measured as soon as possible after processing to avoid any misinterpretations of the intensity of the heat treatment.

Keywords: UHT milk, Lactulose, Furosine, β -lactoglobulin, Bovine serum albumin.

Ultra-high-temperature (UHT) processing of milk destroys virtually all micro-organisms present, both vegetative forms and spores, with any remaining organisms being incapable of growth in the product under normal storage conditions; this ensures that the UHT milk has a long shelf-life without refrigeration (Burton, 1988). However, such heat treatments also give rise to changes in the nutritional and organoleptic properties of the milk (Mauron, 1981; Burton, 1984; Walstra & Jenness, 1984; Schaafsma, 1989; O'Brien, 1995; van Boekel, 1998; Lewis & Heppell, 2000). Furthermore, additional changes such as losses of vitamins, flavour and colour changes, Maillard-type reactions and age gelation can occur in the milk during storage (Burton, 1988; Renner, 1988; Lewis & Heppell, 2000).

A number of chemical and biological indicators can be used to evaluate changes induced during processing and storage of UHT milk. The heat load a milk receives can be estimated by measuring chemical indicators such as lactulose (Andrews, 1986; Berg & van Boekel, 1994; Morales et al. 2000), furosine (Erbersdobler et al. 1987; Resmini et al. 1990; Resmini & Pellegrino, 1994), 5-hydroxymethylfurfural (HMF) (Morales et al. 1995, 2000), undenatured whey proteins (Resmini et al. 1989; Morales et al. 2000) and soluble tryptophan (Birlouez-Aragon et al. 1998). Some of these indicators also change

during storage, limiting their value as heat load indicators in stored milks.

Previously, we have used some chemical indicators to assess heat damage in milks processed on a UHT pilot plant, both immediately after heating and during storage (Elliott et al. 2003). In the present paper, we report the use of such indicators to evaluate the changes in commercial UHT milks, both directly and indirectly heated, during 24 weeks' storage.

Materials and Methods

Milk samples

Multiple packages from single batches of commercially UHT heat-treated whole (33–40 g fat/l), semi-skim (16 g fat/l) and skim (1–3 g fat/l) milk were obtained from seven Australian UHT milk companies (Table 1). Each UHT processor collected samples directly from their processing line and forwarded them immediately to the authors for analysis. The milks were stored at 20 ± 2 °C for 24 weeks and analysed at weeks 1, 4, 8, 12, 16, 20 and 24 for a range of parameters (see below); a single but different package of each milk was opened at each analysis time and analysed in duplicate.

Chemicals and Materials

Chemicals were sourced as outlined by Elliott et al. (2003) except that sodium dihydrogen orthophosphate, disodium

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Table 1. Details of commercial UHT milks examined in this study

Sample no	Company	Milk type	Fat %	Protein %	Heating mode	Sterilisation Temp/time	Packaging	Volume
1	A	FC	3.8	3.5	I	141 °C/4 sec	Tetrabrik	1 L
2		SM	0.1	3.3	I		Tetrabrik	1 L
3	B	FC	3.5	3.4	I	142 °C/6 sec	Tetrabrik	250 ml
4		SM	0.1	3.5	I		Tetrabrik	250 ml
5		FC	3.5	3.4	I	138 °C/6 sec	Tetrabrik	1 L
6		SM	0.1	3.5	I		Tetrabrik	1 L
7		SS	1.6	3.5	I		Tetrabrik	1 L
8		FC	3.4	3.4	I		Tetrabrik	1 L
9	C	FC	3.5	3.4	D	145 °C/5 sec	3LHDPE	1 L
10		SM	0.1	3.5	D		3LHDPE	1 L
11		FC	3.5	3.4	D		3LHDPE	1 L
12		SM	0.1	3.5	D		3LHDPE	1 L
13		FC	3.5	3.4	D		3LHDPE	1 L
14	D	FC	3.5	3.3	I	142 °C/2 sec	Tetrabrik	1 L
15		SM	0.3	3.9	I		Tetrabrik	1 L
16	E	FC	3.3	3.2	I	144 °C/6 sec	Tetrabrik	1 L
17		SM	0.1	3.4	I		Tetrabrik	1 L
18	F	FC	4.0	3.1	D	147 °C/6 sec	Tetrabrik	1 L
19		SM	0.1	4.6	I	143 °C/4 sec	Tetrabrik	1 L
20		FC	3.6	3.1	I		Tetrabrik	250 ml
21	G	FC	3.5	3.2	I	140 °C/4 sec	Combibloc	1 L
22		SM	0.1	3.4	I		Combibloc	1 L

FC=Full-cream milk. SM=Skimmed milk. SS=Semi-skimmed milk. I=Indirect UHT heating. D=Direct UHT heating. 3LHDPE=Three-layer high-density polyethylene plastic-laminate bottle

hydrogen orthophosphate, sodium hydroxide and acetone were from Ajax Chemicals (Auburn, NSW, Australia); α -lactalbumin (α -la), phosphotungstic acid, fluorescamine, zinc acetate dihydrate were from Fluka Chemicals (Castle Hill, NSW, Australia); HPLC-grade acetonitrile was from Labscan (Bangkok, Thailand) and trifluoroacetic acid was from Scharlau (Barcelona, Spain). Strata SPE C₁₈-T 500 mg, 6 ml tubes were from Phenomenex (Pennant Hills, NSW, Australia). Filter papers were from Filtech (Armidale, NSW, Australia). All aqueous solutions were prepared with high-purity water produced with a Millipore Milli Q system. All reagents were of analytical reagent grade unless otherwise stated.

High Performance Liquid Chromatography (HPLC)

All chromatographic separations were carried out on a Shimadzu HPLC system consisting of a Model LC-10AT pump, a FCV-10A low-pressure gradient system, a DGU-10A degasser, a SIL-10AD autoinjector and a CTO-10AS column oven. The sample components were detected with either a SPD M10AV UV/Vis detector equipped with an 8 μ l flow cell or a RID-10A refractive index detector equipped with a 9 μ l flow cell. Instrument control and data acquisition and analysis were carried out with Shimadzu CLASS-VP™ software. All mobile phases were filtered using a Millipore (Milford, MA, USA) system with 0.22 μ m membrane filters (47 mm) and all samples were filtered using a Millex®-GP syringe-driven Millipore filter unit with 0.22 μ m membrane filters (13 mm) prior to injection.

Analytical methods

Lactulose. Lactulose was determined as outlined by Elliott et al. (2003) using a Rezex RPM monosaccharide lead-based cation exchange column (300 \times 7.8 mm I.D.) (Phenomenex, Pennant Hills, Sydney, NSW, Australia).

Furosine. Furosine was determined as outlined by Elliott et al. (2003).

Undenatured whey proteins. The method of Resmini et al. (1989) was used with some modifications. Well-mixed milk (25 ml) was adjusted to pH 4.6 with a 2 M-HCl solution under constant stirring and allowed to stand at room temperature for 15 min for precipitation of casein. After this time, the liquid portion was filtered through Filtech No 1893-150 filter paper and a 0.22 μ m nylon filter (Millex®-GP syringe driven filter unit) before being injected into the HPLC. Separations were achieved on a Jupiter 5 μ m C₁₈ 300 Å column (250 \times 4.6 mm I.D.) (Phenomenex, Pennant Hills, Sydney, NSW, Australia) operating at 40 °C. A binary solvent system operating at 1 ml/min consisted of solvent A: trifluoroacetic acid (0.013 M) in acetonitrile and solvent B: trifluoroacetic acid (0.013 M) in water. The elution gradient programme used throughout the chromatographic run is displayed in Table 2; the total run time for each sample was 60 min. Detection was at 205 nm and the injection volume was 50 μ l. α -La and β -lactoglobulin (β -lg) were quantified using calibration curves for α -la and β -lg in the range

Table 2. Elution gradient profile for whey protein analysis

Time (min)	Solvent A† (%)	Solvent B‡ (%)
0·01	27·0	73·0
2·00	32·0	68·0
36·90	48·4	51·6
40·90	50·2	49·8
43·90	27·0	73·0
60·00	27·0	73·0

† Solvent A = trifluoroacetic acid (0·013 M in acetonitrile)

‡ Solvent B = trifluoroacetic acid (0·013 M in water)

0–2000 mg/l while bovine serum albumin (BSA) was quantified using a calibration curve for BSA in the range of 0–150 mg/l. Milk samples were analysed in duplicate.

Statistical analysis. Statistically significant differences between the directly and indirectly heated UHT milks over the 24 weeks' storage period was evaluated by an analysis of variance (ANOVA) using the General Linear Model Procedure of the SAS software version 6·012 (1996).

Results and Discussion

The data on the milks shown in Table 1 have been collated to allow a comparison of directly and indirectly processed milks. The data for skim, semi-skim and full-cream milks have been combined as they exhibited similar trends for all analytes.

Effect of UHT heating on chemical indices

The data for lactulose and furosine are shown in Table 3 and those for the whey proteins are shown in Table 4. At week 1, it was evident that the indirectly heated UHT milks had sustained more heat damage than the directly heated UHT milks. The levels of lactulose and furosine for the indirect UHT milks were significantly higher (all $P < 0·01$) than those of the directly heated milks, and the undenatured whey proteins (α -la, β -lg and BSA) were lower (all $P < 0·01$) in the indirectly heated milks. This was despite the fact that various temperature–time profiles were used by the manufacturers of each type of UHT milk (Table 1). Nangpal & Reuter (1990a) attributed differences in the effects of direct and indirect processing to the comparatively longer heating and cooling stages associated with indirect heating.

Effect of storage of UHT milks on chemical indices

The levels of the heat indices of the UHT milks are given in Tables 3 & 4. Lactulose did not show a statistically significant change. From previous studies on the behaviour

Table 3. Lactulose and furosine data (mean values \pm standard deviations) for commercial directly and indirectly processed UHT milks during storage at room temperature for 24 weeks

Analyte	Week	Direct ($n=6$)	Indirect ($n=16$)
Lactulose (mg/l)	1	125 \pm 20 ^a	466 \pm 217 ^a
	4	164 \pm 21 ^a	454 \pm 216 ^a
	8	179 \pm 43 ^a	471 \pm 223 ^a
	12	192 \pm 34 ^a	477 \pm 230 ^a
	16	204 \pm 36 ^a	487 \pm 224 ^a
	20	216 \pm 34 ^a	507 \pm 247 ^a
	24	244 \pm 29 ^a	531 \pm 257 ^a
Furosine (mg/100g protein)	1	29 \pm 21 ^a	124 \pm 55 ^a
	4	44 \pm 33 ^a	125 \pm 54 ^a
	8	61 \pm 38 ^b	135 \pm 53 ^b
	12	75 \pm 40 ^c	144 \pm 45 ^c
	16	90 \pm 38 ^d	153 \pm 41 ^d
	20	109 \pm 33 ^e	165 \pm 39 ^e
	24	132 \pm 36 ^f	183 \pm 43 ^f

^{a–f} Values within columns with different superscripts are significantly different ($P < 0·05$).

Table 4. α -Lactalbumin, β -lactoglobulin and BSA data (mean values \pm standard deviations) for commercial directly and indirectly processed UHT milks during storage at room temperature for 24 weeks

Analyte (mg/l)	Week	Direct ($n=6$)	Indirect ($n=16$)
α -Lactalbumin	1	1131 \pm 340 ^a	356 \pm 176 ^a
	4	1069 \pm 328 ^a	351 \pm 181 ^a
	8	1032 \pm 322 ^a	352 \pm 183 ^a
	12	1011 \pm 364 ^a	352 \pm 174 ^a
	16	1072 \pm 381 ^a	346 \pm 169 ^a
	20	1036 \pm 363 ^a	323 \pm 159 ^a
	24	988 \pm 372 ^a	304 \pm 149 ^a
β -Lactoglobulin	1	1404 \pm 516 ^a	190 \pm 52 ^a
	4	1266 \pm 502 ^a	188 \pm 52 ^a
	8	1213 \pm 525 ^a	191 \pm 60 ^a
	12	1172 \pm 520 ^a	191 \pm 52 ^a
	16	1151 \pm 506 ^a	189 \pm 56 ^a
	20	1135 \pm 526 ^a	180 \pm 57 ^a
	24	1126 \pm 517 ^a	164 \pm 59 ^a
BSA	1	96·5 \pm 36·7 ^a	15·8 \pm 10·1 ^a
	4	75·9 \pm 33·7 ^b	13·8 \pm 9·8 ^a
	8	70·1 \pm 32·6 ^c	12·6 \pm 9·6 ^a
	12	53·3 \pm 26·4 ^d	11·4 \pm 7·9 ^a
	16	57·0 \pm 24·5 ^e	9·5 \pm 6·5 ^a
	20	40·7 \pm 20·3 ^f	8·7 \pm 5·3 ^a
	24	32·4 \pm 13·6 ^g	6·2 \pm 4·4 ^a

^{a–g} Values within columns with different superscripts are significantly different ($P < 0·05$).

of lactulose during storage, it is unclear whether a "typical" trend exists for this compound. Andrews (1989) found that the level of lactulose in a range of commercial

Table 5. Values of heat treatment indicators previously reported

Indicator	Milk Type		
	Direct	Indirect	Reference
Lactulose (mg/l)	90–250 99–175	310–570 195–669	(Calvo et al. 1987) (Andrews, 1989)
Furosine (mg/100g protein)	59–167 59–167 50–170	48–294 58–282 150–300	(López-Fandiño et al. 1993) (Corzo et al. 1994b) (Resmini & Pellegrino, 1994)
α -Lactalbumin (mg/l)	220–1036	63–1210	(Andreini et al. 1990)
β -Lactoglobulin (mg/l)	739–792	142–154	(Recio et al. 1996)
BSA (mg/l)	0–131	0–9.5	(Andreini et al. 1990)

UHT milk samples either increased or decreased during six-months' storage at room temperature. In addition, Nangpal & Reuter (1990a) found that lactulose increased during storage at 20 °C as well as at elevated temperatures. However, contrasting these findings, a number of previous studies found the level of lactulose to be largely stable during storage at ~20–25 °C (Andrews, 1984, 1985; Moberg & Hegg, 1985; Corzo et al. 1988; Akalin & Göncü, 1997; Elliott et al. 2003) or only shows an increase with storage time when stored above this temperature (Jiménez-Pérez et al. 1992; Akalin & Göncü, 1997). The conflicting reports arise because, during storage, lactulose continues to be formed, but at the same time is also degraded (Andrews, 1989). The resultant trend depends on extrinsic factors such as heating and storage temperatures, and intrinsic properties of the milk such as pH during storage.

During storage, the furosine content increased significantly for both direct and indirect UHT-heated milks (Table 5). This trend has also been observed in other studies of UHT milk (Nangpal & Reuter, 1990b; Corzo et al. 1994a; Evangelisti et al. 1999). The increase is caused by a slow Maillard reaction, which continuously forms lactulosyllysine. Although both milks displayed an increase in furosine with storage time a larger increase occurred for the indirect milks compared to the direct samples. As indirect milk is more severely heated, the Maillard reaction would be further advanced compared to the directly heated UHT milk and would be expected to progress faster during storage.

The average levels of acid-soluble whey proteins, α -la, β -lg and BSA during the 24 weeks' storage of the direct and indirect UHT milks are given in Table 4. Due the large variability in the data for α -la, β -lg and BSA, only the change in BSA for the direct UHT milks was statistically significant. The HPLC peaks for α -la and β -lg, from which the data for these components were derived, changed in shape during storage and this may have contributed to the large variability in the data. The changes may result from a number of different factors including the formation of lactose adducts through the Maillard reaction (Leonil et al. 1997; Siciliano et al. 2000) or complexes with other proteins (Recio et al. 1996).

Comparison with reported values

Comparison of the values of the heat treatment indicators obtained in this work with those reported for other UHT milks (Table 5 and Elliott et al. (2003)) indicates that the Australian UHT milks had undergone similar amounts of heat treatment to their counterparts in other countries. However, as previous work in this laboratory has shown that the age of the milk at processing and the time of storage after processing can affect some indicators (Elliott et al. 2003), direct comparison of the present values with those previously reported is difficult. The comparison is further complicated by the fact that wide variations exist within and between reports due to different analytical methodologies employed, and different raw milk compositions and qualities.

IDF/EC processing estimation values

Both the International Dairy Federation (IDF) and European Commission have proposed that lactulose and acid-soluble whey proteins be used to distinguish UHT milk from in-bottle sterilised milk (EC, 1992; IDF, 1993). To be classed as UHT milk, milks must have a lactulose content between 100 mg/l and 600 mg/l while the β -lg content must be between 2000 mg/l and 50 mg/l. The wide difference in limits for each compound allows for the large variation in UHT heating conditions employed and changes occurring during storage; this is consistent with the data obtained in this study.

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