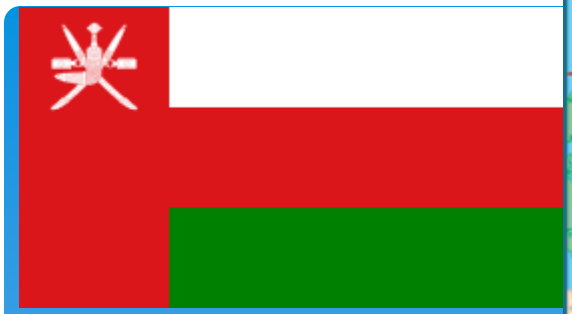

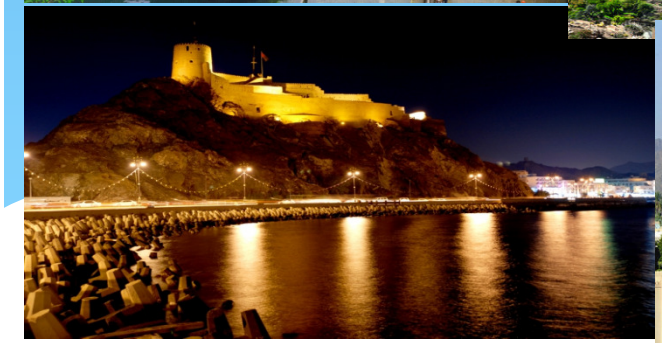


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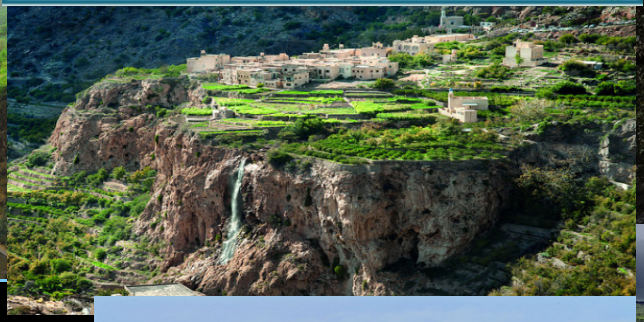
66°30'N
ASIA
EUROPE
Black Sea
Caspian Sea
Aral Sea
Med. Sea
MIDDLE EAST
Oman
TROPIC OF CANCER
23°5'N
Red Sea
Arabian Sea
Bay of Bengal
Pacific Ocean
OCEANIA
AFRICA
Gulf of Aden
0°
EQUATOR
Indian Ocean
TROPIC OF CAPRICORN
23°5'S
AUSTRALIA
1500 mi
1500 km
worldatlas

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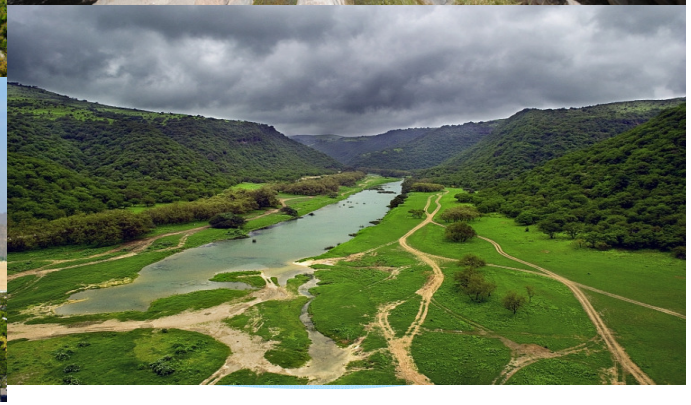

Effect of Hydrogen Peroxide on Volatile Sulfur Compounds in UHT Milk

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Outline

* **Introduction**
* **Objectives**
* **Analysis**
* **Results & Discussion**
* **Conclusion**

Introduction

* Cooked flavour in UHT milk is linked to the volatile sulfur compounds (VSCs) , denatured β-lactoglobulin (β-Lg)
* VSCs (DMS, DMDS, CS_2, MeSH, COS, H_2S, DMTS,DMSO,Me_2SO_2)
* This off-flavour limits the acceptance of UHT milk
* Several additives tested (e.g copper, calcium chloride, disodium hydrogen phosphate, sodium iodate/bromate)
* Not approved by food regulatory agencies in many countries
* May impart undesirable flavours to milk

Introduction

* **Why Hydrogen peroxide (H_2O_2) ?**
* **H_2O_2 is an effective milk preservative on its own & in combination with heat (McDoneell *et al.* 2006)**
* **H_2O_2 is a component of LP-system**
* **Addition of H_2O_2 to raw milk, approved by the Codex Alimentarius Commission in 1991 (FAO/WHO 1991)**

Introduction

* In the USA, H_2O_2, (GRAS).
* It can be added to;
- milk (at 0.05%) to make certain types of cheese,

- whey (at 0.04%) & starch (at 0.15%),

- wine and instant tea (Sofos and Busta 1999)

* FAO permits the addition of H_2O_2 to milk at 0.05%-0.25% (500-2500mg/L)

- all the H_2O_2 remaining in the milk after processing must converted by catalase into oxygen and water (Tarhan 1995).

Introduction

* **In Australia;**

- **H_2O_2 is an approved processing aid in food with a maximum permitted residue level of 0.005% (50 mg/L) in the final food (FSANZ 2006).**

* **H_2O_2, sterilizing food packaging materials including UHT milk packages.**

* **FDA, limits H_2O_2 residues to 0.00005% (0.5 mg/L)) in the finished food package (CFR 2003).**

Objectives

* **Aims/study;**

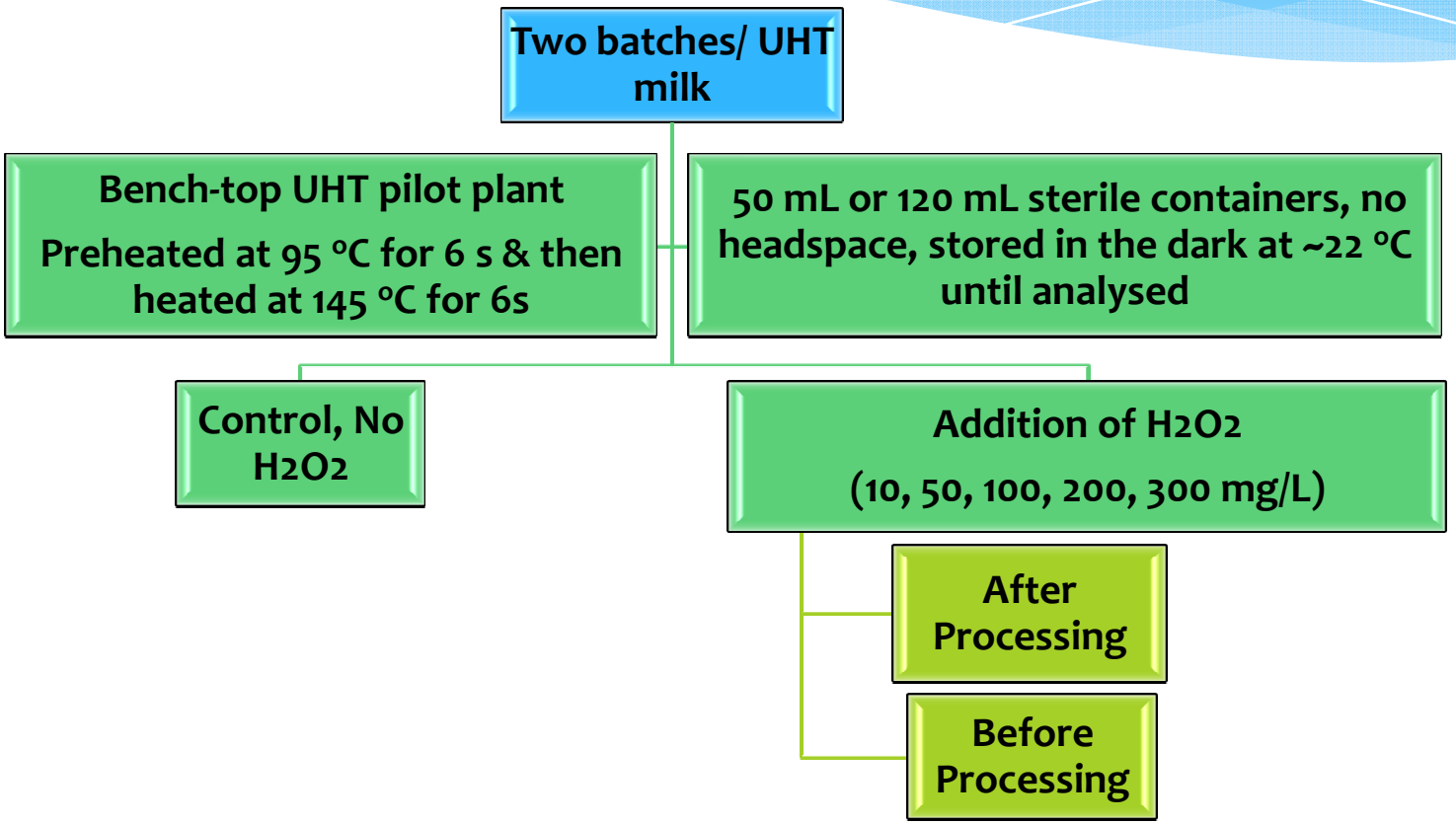
- **the effectiveness of using H2O2 (10, 50, 100, 200, 300 mg/L) in reducing the levels of VSCs in UHT milk,**

- **Its effect on β- lactoglobulin denaturation in UHT and batch-heated milk**

Analysis

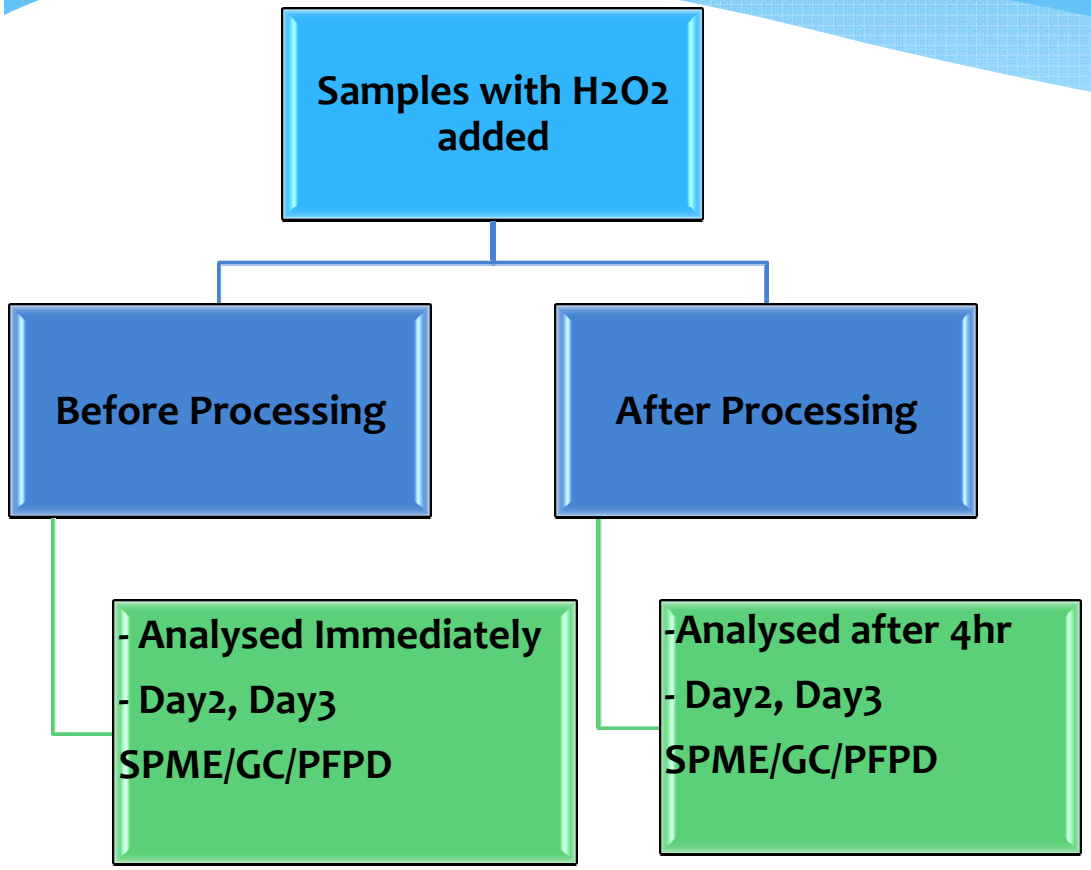
A. Effect of H_2O_2 on Sulfur Volatiles

Milk sample preparation and H_2O_2 addition



Bench-top UHT pilot plant

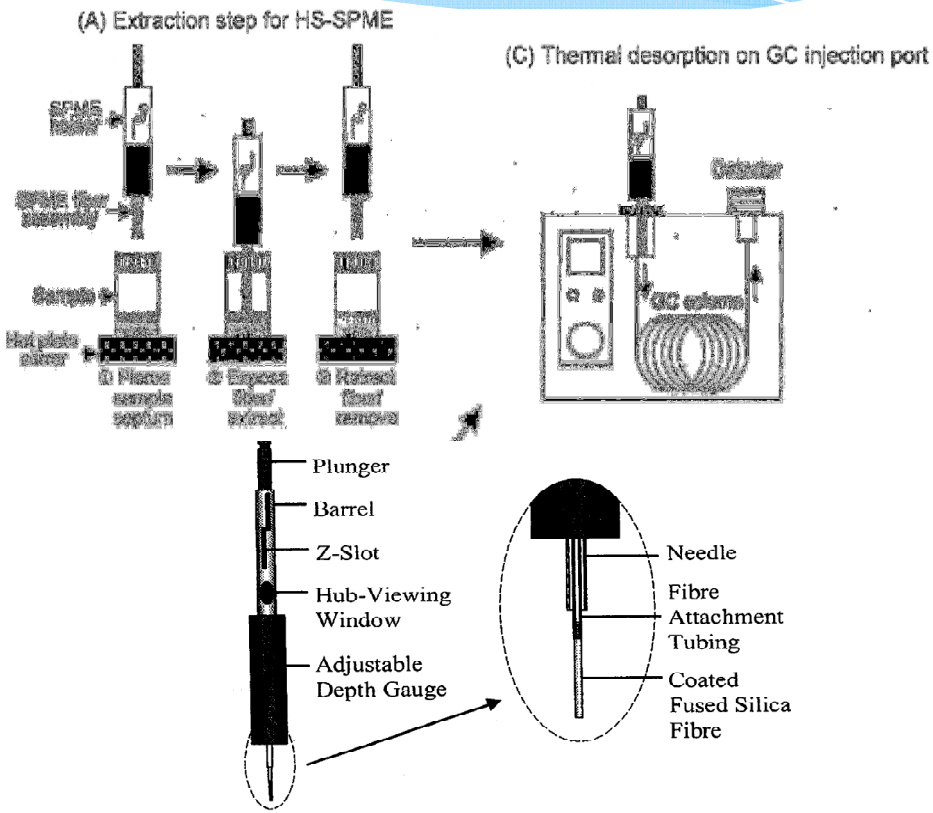
A. Effect of H2O2 on Sulfur Volatiles



* H_2O_2 residues measured semi-quantitatively at RT for up to one week after processing using Merckoquant® 126 Peroxide strips (Merck, Australia).
* The colour chart referred to concentration ranges of 1-3-10-30-100 $mg.L^{-1}$

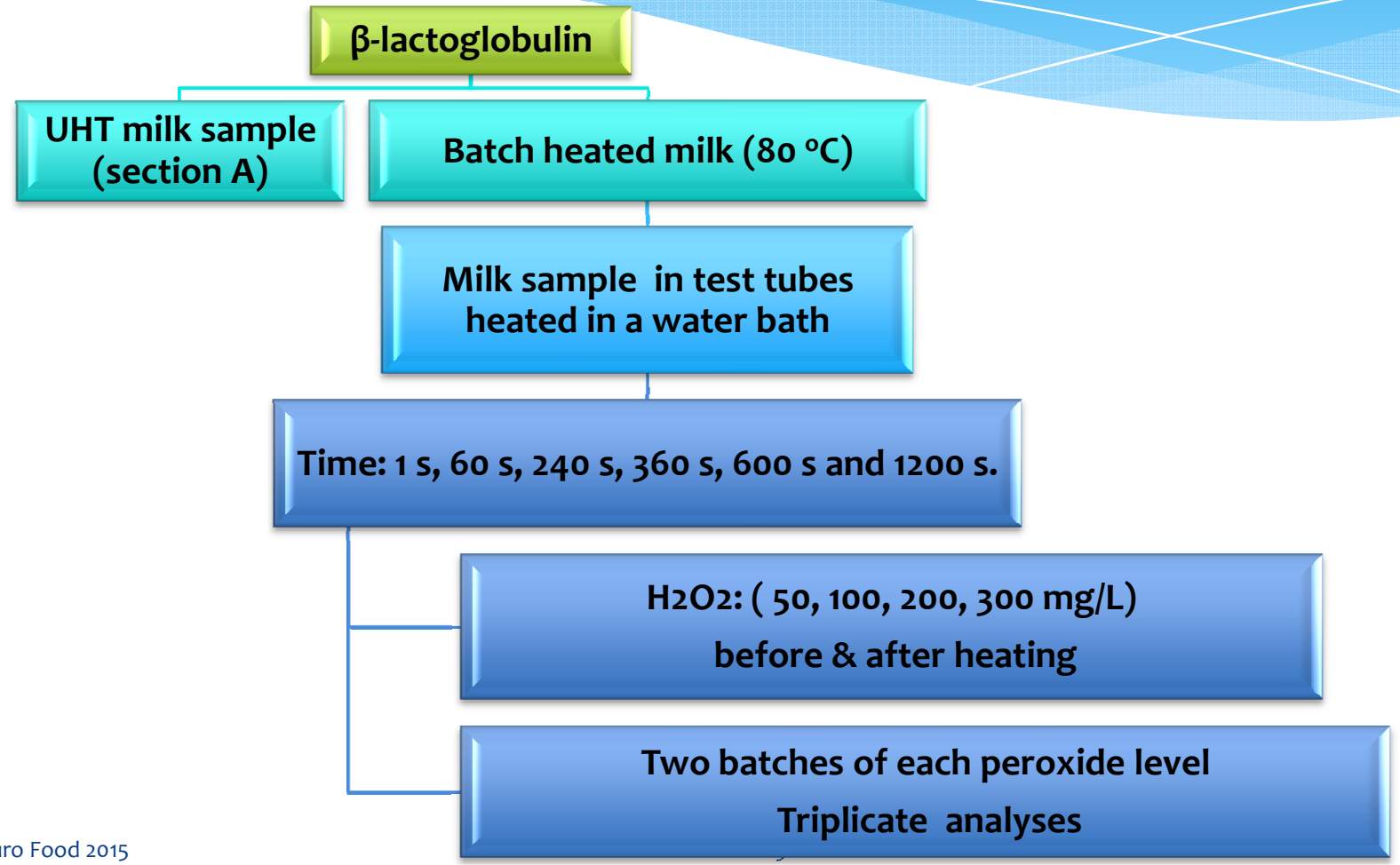
Analysis For Volatile sulfur compounds

* SPME/GC/PFPD
* GC: Varian CP-3800
* **Sample**: 5 mL , 10 mL screw-top vial, fitted with a PTFE-faced silicone septum.
* **Fiber**: CAR-PDMS, 85 µm (Supelco, Australia)
*
* **Extraction time & temperature**: 30 °C /15 min.
* Triplicate analyses



Analysis

B. Effect of H_2O_2 on β-lactoglobulin using UHT & Batch-heated Milk



Analysis For β-lactoglobulin Denaturation

- analysis was performed according to Elliott *et al*. (2005)
- Sample (1ml)
- Eppendorf tube

- 30 µL of acetic acid (33%) added
- stand for 10 min
- 30 µL of 3.3 M sodium acetate
- centrifuged for 20 min at 4500 rpm
- supernatant removed carefully and filtered through a 0.22 µm Millex filter (Millipore Corporation, Bedford, USA).

- Shimadzu HPLC system with a SPD M10AV UV/Vis detector operated at 205 nm.
- standard of β-lactoglobulin
- Triplicate analyses

Results & discussion

A. The effects of H_2O_2 addition on volatile sulfur compounds

Table 1. Volatile sulfur compounds (µg L^{-1}) in UHT milk, with H_2O_2 added before and after processing, during 3-d storage

a. H_2S (µg L^{-1})

H_2O_2 (mg L^{-1})	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0	14.73±3.68	0.89±0.09	0.70±0.09	14.7±3.68	0.89±0.09	0.70±0.09
10	4.06±2.02	0.68±0.12		0.70±0.09	0.59 ±0.00	
50						
100						
200						
300						

- ✓ Day1, completely oxidized when added either before or after processing, at all concentrations except 10 mg/L H2O2 (Conc. below its threshold value (10µg/L).
- ✓ H2O2 was more efficient in oxidizing H2S when added at 10 mg/L after processing

b. COS (mg L^{-1})

H_2O_2 (mg L^{-1})	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0	1.07±0.27	0.25±0.01	0.11±0.00	1.07±0.27	0.25±0.01	0.11±0.00
10	0.37±0.16	0.26±0.15	0.08±0.02	0.28±0.13	0.12±0.05	0.07±0.01
50				0.09±0.01		
100				0.05±0.01		
200						
300						

- Completely oxidized when it was added before processing at all concentrations, except for 10 mg/L H2O2 (Day1) .
- After processing, COS decreased from 0.28 mg/L at 10 mg/L H2O2 to 0.05 mg/L at the 100 mg/L H2O2
- The addition of 50 mg/L and 100 mg/L H2O2 caused the complete disappearance of COS by day2 when added after processing.

c. MeSH ($\mu g\ L^{-1}$)

H_2O_2 ($mg\ L^{-1}$)	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0	2.33±0.04	0.97±0.05	0.51±0.00	2.33±0.04	0.97±0.05	0.51±0.00
10	1.47±0.12	0.62±0.08		2.12±0.34	0.78±0.05	
50	1.47±0.20			0.90±0.01		
100	1.17±0.17			0.53±0.04		
200	0.23±0.05			0.25±0.07		
300						

- Conc. higher than its threshold value in water (0.2µg/L)
- Undetectable at 300 mg/L H2O2. on day one
- MeSH conc. Gradually ↓with↑ H2O2 conc. added either before or after processing
- Completely oxidized by day two for both additions (before or after), except in the 10 mg/L H2O2 samples where it disappeared by day three.
- The oxidation rate of MeSH was slightly higher when H2O2 was added after processing.

d. DMS (µg L^{-1})

H_2O_2 (mg L^{-1})	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0	8.54±0.86	6.26±0.50	4.57±0.22	8.54±0.86	6.26±0.50	4.57±0.22
10	7.66±1.33	6.25±0.07	5.15±0.41	6.68±1.33	6.14±2.14	4.32±1.02
50	6.40±0.60	4.25±1.30	3.07±0.80	4.93±0.07	3.50±0.44	2.70±0.63
100	4.94±0.12	2.25±0.32	1.85±0.01	3.12±0.32	1.23±0.42	1.11±0.11
200	2.24±0.32	0.48±0.05	0.44±0.04	1.02±0.38		
300	1.22±0.40			0.52±0.11		

* DMS conc. in control sample was 8.54 µg/L , < threshold values in milk, 20 µg/L.
* The DMS conc. gradually decreased as the H2O2 conc. increased, added either before or after
* The conc. similar in the control sample and the samples with 10 mg/L peroxide even during storage indicating limited oxidation of DMS at low H2O2 concentration.
* The oxidation rate of DMS was slightly higher when H2O2 was added after processing.

e. CS_2 (µg L^{-1})

H_2O_2 (mg L^{-1})	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0	3.29±0.95	1.96±0.40	1.91±0.23	3.29±0.95	1.96±0.40	1.91±0.23
10	3.25±0.69	4.22±1.62	3.0±0.55	2.53±0.49	2.11±0.34	1.69±0.35
50	1.04±0.20	0.81±0.20	0.62±0.20	2.09±0.01	1.43±0.30	1.18±0.25
100	0.61±0.15	0.46±0.11	0.23±0.03	1.96±0.13	1.44±0.15	1.35±0.08
200	0.38±0.04			1.95±0.16	1.36±0.14	1.23±0.06
300	0.32±0.00			1.87±0.03	1.33±0.24	1.1±0.14

* The CS2 conc. control sample was 3.29 µg/L , < threshold values in milk 100-1000 µg/L
* H2O2 was more effective in oxidizing CS2 when added before processing than after processing
* At the 200 mg/L & 300 mg/L H2O2 concentrations, CS2 was undetectable on days two and three.

f. DMDS (µg g L^{-1})

H_2O_2 (mg L^{-1})	H_2O_2 added before processing			H_2O_2 added after processing		
	1 d	2 d	3 d	1 d	2 d	3 d
0						
10						
50						
100	0.06±0.02	0.13±0.02	0.12±0.00			
200	0.16±0.02	0.18±0.01	0.18±0.01			
300	0.19±0.01	0.17±0.00	0.17±0.00			

* Not detected in the control UHT milk
* Produced when the H2O2 was added before processing at conc. of 100, 200 and 300 mg/L
* Conc. below the threshold value in milk (21µg/L)
* It is presumed that the DMDS was formed from MeSH through oxidation by H2O2.
* No DMDS was generated when the peroxide was added after processing.

Table 3. H_2O_2 residues (mg L^{-1}) in UHT milk following addition before and after processing

H_2O_2 added (mg L^{-1})	H_2O_2 added before heating, measured on						H_2O_2 added after heating, measured on					
	0 h	1 d	2d	3 d	4 d	7 d	4 h	1 d	2d	3 d	4 d	7 d
10							1					
50	10	1					30	6	1			
100	40	4	1				50	30	3	2		
200	120	40	20	6	3		150	40	30	30	20	9
300	150	60	40	30	60	30	210	150	100	90	90	90

- ✓ **The residues detected when the H2O2 was added after processing were higher than when H2O2 addition was made before processing**
- ✓ **This can be attributed to the heat treatment, since H2O2 can be removed from milk by heating and prolonged storage (Mishra *et al.* 1985).**

B. The effects of H2O2 on whey proteins
(A) UHT Milk

Table 4 Whey protein denaturation in UHT milk with and without H2O2 added before and after processing.

Milk sample	β-lactoglobulin denaturation (%)		α-lactoalbumin denaturation (%)	
	H_2O_2 added before	H_2O_2 added after	H_2O_2 added before	H_2O_2 added after
UHT control	95.9	95.9	78	78
UHT + 10 mg/L H_2O_2	94.2	95	63	73
UHT + 50 mg/L H_2O_2	99.3	97.8	78	83
UHT + 100 mg/L H_2O_2	99.6	97.7	89	85
UHT + 200 mg/L H_2O_2	99.5	98.1	100	96
UHT + 300 mg/L H_2O_2	100	97.9	100	100

Overall, as for β-lactoglobulin, α-lactalbumin was denatured more when H2O2 was added before processing than when added after processing.

B. The effects of H2O2 on whey proteins
(B) Batch-Heated Milk

Table 5 β-Lactoglobulin denaturation (%) in batch-heated milk without and with H2O2 added before and after processing

Processing Conditions	Control No H_2O_2	50 mg/l H_2O_2		100 mg/L H_2O_2		200 mg/L H_2O_2		300 mg/L H_2O_2	
		Before	After	Before	After	Before	After	Before	After
80 °C, 1 s	40	30	40	55	32	58	41	70	45
80 °C, 60 s	49	42	47	62	43	65	46	73	53
80 °C, 240 s	69	63	70	72	70	80	72	87	73
80 °C, 360 s	79	72	77	80	77	88	80	94	83
80 °C, 600 s	89	84	90	88	91	89	90	97	92
80 °C, 1200 s	98	96	97	96	98	98	98	99	98

The addition of H2O2 after heating caused less denaturation at the shorter holding times (i.e. 1 s & 60 s) than addition before heating except at 50 mg/L H2O2 where it had minimal effect at all holding times as well as less denaturation compared to the control

Conclusion

* **Low concentrations of H_2O_2 (10 mg/L or 50mg/L) were sufficient to reduce the level of VSC in the UHT milk under the processing conditions used.**

* **One of the major contributors to the cooked flavour, H_2S, was completely eliminated or reduced to well below its flavour threshold value.**

* **Low conc. of H_2O_2 had no effect on, or reduced, β-lactoglobulin denaturation when added after or before processing, respectively.**

* **The addition of H_2O_2 could be a practical solution to the prevention or alleviation of cooked flavour development in UHT milk**

* **However, the levels of H_2O_2 addition must be within the range permitted by the relevant regulatory jurisdiction.**