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Sensory evaluation and storage stability of UHT milk fortified with iron, magnesium and zinc

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Abstract Fortification of staple foods is an effective means of addressing dietary deficiencies of iron, magnesium and zinc, which are common throughout the world. In this work, homogenized whole milk was fortified with iron, magnesium and zinc, individually and collectively, shortly before ultra-high-temperature (UHT) processing (145 °C, 6 s). Sulphates of iron, magnesium and zinc were added to give total concentrations (natural plus added) of approximately 25, 50, 75 and 100% of the recommended daily intakes (RDI) per litre of the UHT milk. The highest concentrations (100% RDI) were 8, 320 and 16 mg.L\(^{-1}\) for iron, magnesium and zinc, respectively. The fortified milks were analysed to determine the distribution of the minerals, their sensory characteristics and their stability during storage. Zinc and iron preferentially became associated with the casein fraction, while most of the magnesium remained in the serum phase. Some sensory panellists could perceive a different taste in UHT milk supplemented to 75% of the RDI per litre, but not in milks supplemented to 50%. During storage at 30 °C of milks supplemented to 50%, changes in flavour were detected in milks supplemented with zinc and iron after 30 and 60 days, respectively; this generally corresponded with increases in oxidation. Viscosity and protein hydrolysis also increased but not to detrimental levels. It was concluded that fortification of UHT milk with magnesium and possibly zinc has considerable potential, but fortification with iron is more challenging due its ability to catalyse oxidation.

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Keywords UHT milk · Fortification · Iron · Magnesium · Zinc · Sensory

1 Introduction

Inadequate intake of the essential micronutrients, iron (Fe), magnesium (Mg) and zinc (Zn), is common in many countries. This is particularly important for women (Yang and Huffman 2011), and infants and young children (Krebs et al. 2006). The inadequate intake is exacerbated in many cases by the presence in the diet of mineral-binding substances such as phytate and oxalate.

Iron is essential for many metabolic processes in the human body, and two thirds of it is associated with haemoglobin in the red cells of the blood. Low levels in the blood cause iron deficiency anaemia which is one of the most widespread disorders in both developed and developing countries (Stolzfus 2003; Toxqui et al. 2013). WHO has estimated that anaemia, whose primary cause is iron deficiency, affects 52% of preschool-age children, 34% of pregnant women and 44% of non-pregnant women in the world (de Benoist et al. 2008). A high incidence occurs in African and Asian populations. In the USA, Killip et al. (2007) reported that 2% of adult men were iron deficient but the prevalence in women was much higher, up to 20% in black and Mexican-American women. USDA (2009) estimated about 10% of Americans consumed less than the recommended daily intake (RDI) of iron. Low iron status has been linked to several diseases in women in the post-menopausal period (Bendich and Zilberboim 2010).

Magnesium is necessary for healthy bone development and maintenance (Abrams et al. 2014). About half of the approximately 25 g of magnesium contained in a human body is in bones (Hunt and Nielsen 2009). It has been suggested that magnesium may be even more significant than calcium in bone development (Abrams et al. 2014) and in the bone disorder, osteoporosis (Abraham and Grewal 1990). USDA (2009) estimated that 57% of the US population may have an inadequate intake of magnesium.

Zinc is involved in many body activities such as protein and nucleic acid biosynthesis, carbohydrate and lipid metabolism, immunity, growth and fertility (Samman 2007; Prasad 2009). The adult human body contains 1.5–2.5 g of zinc, 95% of which is intracellular. Wessells and Brown (2012) estimated that around 17% of the world’s population may have inadequate zinc intake while USDA (2009) estimated that 29% of Americans may be zinc deficient.

Therefore, there is a need for fortification of foods with Fe, Mg and Zn to alleviate deficiencies in these elements. The increasing worldwide demand for ultra-high-temperature (UHT)-processed milk (Chavan et al. 2011) makes it an ideal product for such fortification, particularly as it naturally contains relatively low levels of these elements. UHT milk, which is processed at 135–145 °C for 1–10 s, has a shelf life at room temperature of 6 months or more and hence can be distributed widely without refrigeration.

Milk naturally contains low levels of Fe, 0.20–0.5 mg.L\(^{-1}\) (Fransson and Lonnerdal 1983; Holt 1997; Hunt and Meacham 2001), and Zn, 3–5 mg.L\(^{-1}\) (Hunt and Meacham 2001), and moderate amounts of Mg, 110 mg.L\(^{-1}\) (Holt 1997). Most of the Fe and Zn is in the colloidal phase with the Zn being mostly associated with the colloidal calcium phosphate and the Fe being bound to the polypeptide chains of the caseins (Silva et al. 2001). By contrast, approximately two thirds of the Mg resides in the milk serum (Gaucheron 2011; Tsioulpas et al. 2007). The Mg in the colloidal phase is associated
with the colloidal calcium phosphate. The RDIs for adults for Fe, Mg and Zn according to various sources are around 8–15 mg, 280–420 mg and 11–16 mg, respectively.

UHT milk undergoes several changes during storage (Burton 1988). These include changes in viscosity which can lead to gelation (Kocak and Zadow 1985), proteolysis due to milk plasmin and bacterial proteinases which results in increases in non-casein nitrogen (NCN) (López-Fandiño et al. 1993; Valero et al. 2001) and changes in flavour due, in part, to lipid oxidation (Perkins et al. 2005) which can be accelerated by metals such as copper and iron.

The aim of this work was to fortify UHT milk with Fe, Mg and Zn so that each litre contributed significantly to the RDI of each element. The sensory characteristics and storage stability of UHT-processed fortified milk was investigated. The distribution of the added minerals among the serum, colloidal (micellar casein) and lipid phases of the UHT milk was also investigated to determine whether the added minerals distribute in the same manner as the natural minerals.

2 Materials and methods

2.1 Experimental plan

The work was carried out in three parts:

1. Sensory evaluation of UHT milk fortified with iron, magnesium and zinc to 75% RDI per litre. Three separate batches of milk were processed and evaluated.
2. Distribution of the elements in UHT milk fortified with iron, magnesium, zinc and all three elements to 25, 50, 75 and 100% RDI per litre. The distributions were compared with that in unfortified UHT milk. Three separate batches of milk were processed.
3. Storage trial of UHT milk fortified with iron, magnesium and zinc to 50% RDI per litre at 30 °C for 60 days. Sensory evaluation and physico-chemical analyses (viscosity, lipid oxidation, proteolysis) were performed. Three separate batches of milk were processed.

2.2 Milk

Nine different batches of homogenized, pasteurized whole bovine milk were collected from a local dairy processor immediately after production. The concentrations (% mean ±SD, n=9) of the milk components, determined by infrared analysis, were the following: total solids, 12.6±0.5; fat, 3.89±0.18; protein, 3.27±0.12 (casein, 2.68±0.11; whey proteins, 0.58±0.02); lactose, 4.67±0.17 and ash, 0.75±0.03 (by difference).

2.3 Minerals

Analytical grade FeSO₄·7H₂O, MgSO₄ and ZnSO₄·H₂O were obtained from Sigma-Aldrich (Sydney, NSW, Australia). Depending on the recommended daily intake (RDI) for adults, fortified milk samples with different levels were prepared as shown in Table 1. The RDI values of iron, magnesium and zinc adopted were 8, 320 and...
In preparing the magnesium-fortified milk samples, the amount of magnesium in normal bovine milk (~120 mg.L\(^{-1}\)) was taken into consideration. Trisodium citrate (dihydrate) was added to the milk samples fortified with magnesium to prevent fouling in the UHT plant (Boumpa et al. 2008; Datta and Deeth 2007; Prakash 2007). All other reagents used were of the highest purity available and at least analytical grade.

### Table 1  Mineral salts added (mg.L\(^{-1}\)/mM) to homogenized whole milk

<table>
<thead>
<tr>
<th>Fortification (% RDI)</th>
<th>FeSO(_4)·7H(_2)O</th>
<th>MgSO(_4)</th>
<th>ZnSO(_4)·H(_2)O</th>
<th>Trisodium citrate*</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>9.9/0.04</td>
<td>0</td>
<td>10.9/0.06</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>19.9/0.07</td>
<td>198/1.64</td>
<td>21.9/0.12</td>
<td>322/1.1</td>
</tr>
<tr>
<td>75</td>
<td>29.8/0.11</td>
<td>594/4.94</td>
<td>32.9/0.18</td>
<td>968/3.3</td>
</tr>
<tr>
<td>100</td>
<td>39.8/0.14</td>
<td>990/8.22</td>
<td>43.9/0.24</td>
<td>1,612/5.5</td>
</tr>
</tbody>
</table>

* Added to samples fortified with magnesium  
* Corresponds to ~8 mg.L\(^{-1}\) Fe  
* Corresponds to ~320 mg.L\(^{-1}\) Mg  
* Corresponds to ~16 mg.L\(^{-1}\) Zn

16 mg, respectively. In preparing the magnesium-fortified milk samples, the amount of magnesium in normal bovine milk (~120 mg.L\(^{-1}\)) was taken into consideration. Magnesium can interfere with the performance of the UHT plant (Boumpa et al. 2008; Datta and Deeth 2007; Prakash 2007). All other reagents used were of the highest purity available and at least analytical grade.

#### 2.4 Mineral fortification of milk

Accurately weighed amounts of the salts were thoroughly dissolved in 25 mL of high-purity Milli-Q water per each litre of milk. Fortified whole milk samples were prepared by adding the dissolved salts into the milk and mixing gently shortly before heat treatment. Only 25 mL of distilled water was added to the control milk sample.

#### 2.5 UHT processing

UHT processing was conducted in an indirect (tubular) bench-top UHT plant at the University of Queensland as described by Prakash et al. (2010). The temperature–time conditions in the preheating and high-temperature sections were 95 °C for 8 s and 145 °C for 6 s, respectively. The UHT-processed milks were filled aseptically into gamma ray pre-sterilized polyethylene terephthalate (PET) containers in a laminar flow cabinet sterilized by ultra-violet light (UV) shortly before processing and filling. For the keeping quality trials, all samples were placed in incubators at 30 °C for 60 days.

#### 2.6 Sensory evaluation

Panellists  Sensory evaluation of UHT milk samples was conducted by ten trained panellists (UQ students and staff, seven males, three females, age 20–50 years, healthy subjects, lactose tolerant) selected after completing scaling exercise and basic taste and aroma exercises as per ISO 22935 and trained for 48 h for QDA® of pasteurized and UHT milk. The evaluation was conducted in individual booths in the Food Sensory Laboratory. This study is covered by the Human Ethics Approval #2010000300.
Sensory evaluation test Triangle tests were performed to determine if addition of minerals contributed to perceivable differences in colour, odour and taste of the UHT milk samples. The fortified samples were compared with unfortified UHT milk. Three samples were presented simultaneously to panellists, two from one formulation (either fortified or unfortified UHT milk) and one from a different formulation (either fortified or unfortified UHT milk) in a balanced random order. Panellists were asked to identify the odd sample. To improve the power of the analysis and to be able to detect true discriminators, duplicate triangle tests were conducted for each batch. The data was analysed using chi-square distribution.

The sensory evaluation was carried out in two stages:

Stage 1: In the first stage, UHT milk samples with 75% of the RDI of iron, magnesium and zinc stored refrigerated for 10 days after processing were evaluated for colour, aroma and taste. The panellists evaluated two different batches of UHT fortified milks over two sessions. In each session, duplicate sets of samples were evaluated for colour, aroma and taste.

Stage 2 (storage trial): In the second stage, UHT milk samples with 50% of the RDI of iron, magnesium and zinc were evaluated by the trained panel for taste. Soon after processing, the samples were stored refrigerated for 7 days to allow the sulphury flavour to dissipate. Sensory evaluation was performed on these samples after 7, 30 and 60 days of storage at 30 °C.

2.7 Physico-chemical analyses

2.7.1 Distribution of minerals

The skim milk and milk fat fractions were obtained from whole homogenized milk by centrifuging at 7,000×g and 20 °C for 12 min, using a Beckman Coulter centrifuge (JA-12 rotor, Beckman, USA). The skimmed milk was carefully removed. Sodium azide was added as preservative (0.1 g.L\(^{-1}\)) before storage at 5 °C until analysis.

Ultracentrifugation was used to study the minerals distribution (de la Fuente et al. 1996) between the soluble and micellar casein phases. The skim milk samples were centrifuged at 100,000×g and 20 °C for 2 h, using a Beckman Coulter, Optima L-100 XP Ultracentrifuge (Ti 50.2 rotor, Beckman, USA). The supernatant was carefully removed from the pelleted casein and filtered through Whatman 40 paper and stored at 5 °C until analysis.

Samples of whole milk, skim milk and soluble phase of the milks were prepared for initial digestion for 20 min by addition of 6 and 2 mL of nitric and hydrochloric acids per gramme of sample, respectively. After cooling, 10 mL of triplicate deionized water (TDI) was added to the each sample before digestion in a microwave digester (CEM MSP 1000) for 20 min under pressure of 120 lb per square inch. Hydrogen peroxide (30%, 10 mL) was added to the cooled samples and digested again. Finally, the samples were placed into 25-mL volumetric flasks and the volume was made up with TDI. The concentrations of the mineral elements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). All analyses were performed in duplicate.
2.7.2 Viscosity

The viscosity of control UHT milk and fortified milks were measured at 20 °C using a Brookfield DV-II+ viscometer. A UL adapter (ULA) was used for the determination of viscosity of milk, and the spindle speed was set at 60 rpm. Readings were taken after the spindle had been rotating for 30 s, and the mean of three readings was recorded. The viscosity was expressed in millipascal-second. The tests were done at 0, 15, 30, 45 and 60 days of storage period.

2.7.3 Lipid oxidation

The concentration of thiobarbituric acid reactive substances (TBARS) was determined according to King (1962). The tests were done at 0, 10, 20, 30, 40, 50 and 60 days of storage, and the average of three readings was recorded. A standard curve was prepared with 1,1,3,3-tetraethoxypropane (TEP) as the reactive substance as described by King (1962). The standard curve equation obtained was the following: \( y = 0.119x - 0.0007 \).

2.7.4 Proteolysis

The trinitrobenzene sulphonate (TNBS) method was used for measuring the level of proteolysis (McKellar 1981). The tests were done at 0, 12, 24, 36, 48 and 60 days of storage, and the average of three readings of each sample was recorded. A standard curve was prepared with glycine as the reactive substance as described by McKellar (1981). The standard curve equation obtained was: \( y = 0.208x - 0.0029 \).

2.8 Statistical analysis

All statistical analyses on the physio-chemical data were performed using Statistical Analysis Systems (SAS) software (2002, v.9). The general linear model analysis was performed with the GLM ANOVA tests procedure. When the main ANOVA tests were significant, multiple comparisons of treatments were conducted with the Duncan test.

For the sensory evaluation, duplicate sets of triangle tests were presented in a randomized balanced order and assessed by the trained sensory panel in the same session. The data were analysed using chi-square distribution. A \( P \) value <0.05 indicates a significant difference.

3 Results and discussion

3.1 Distribution of iron, magnesium and zinc in milk components

3.1.1 Iron

The average concentration of natural iron in the milk samples was 0.26 mg.L\(^{-1}\). The distribution of iron among the serum, casein and fat phases in the control and iron-fortified milk samples is shown in Table 2. The levels were highest in the casein...
fraction, and these increased to a plateau level of 82–89% with fortification up to ~50% RDI per litre, much higher than in the control, 60.9%. Concomitantly, there was a marked decrease in the percentage in the fat phase compared with the control. The distributions in the milk samples fortified with all three minerals were similar to those in the samples fortified with iron only. Silva et al. (2001) reported that iron binds to the polypeptide backbone of caseins in the micelle; this contrasts with calcium, magnesium and zinc which are associated with the colloidal calcium phosphate. It is therefore apparent that added iron also shows a high affinity for the proteins in the casein micelle. This is supported by the work of Raouche et al. (2009) which showed the casein micelle to be a good carrier of iron. They reported 84–91% of added iron (up to 20 mM) became associated with the casein micelle. The iron-fortified micelles with up to 15 mM added iron were stable to heat.

### 3.1.2 Magnesium

The average concentration of natural magnesium in the milk samples was 114.9 mg.L\(^{-1}\) (4.59 mM). Since the RDI for magnesium is 320 mg, no magnesium was added for the 25% RDI per litre samples but was added for the 50% and higher samples. Samples with added magnesium caused severe fouling of the UHT plant. This was overcome by addition of low levels of trisodium citrate to chelate the magnesium ions.

Processing of the fortified milk samples did not change the general distribution of magnesium in the milks. It preferentially resides in the milk serum (62.8%) of unfortified milk which contrasts with the other major bivalent ion in milk, calcium (Gaucheron 2011). The proportion of magnesium in the serum phase increased with increasing level of fortification (Table 3). In milk fortified with all three elements, the magnesium distribution was similar with 66.3 to 77.1% being present in the serum. Most likely, most of magnesium was present as a citrate salt since trisodium citrate was added as a stabilizer (Flynn and Cashman 1997).

These results agree with that of de la Fuente et al. (2004) who found 75% of magnesium in milk fortified with 450 mg.L\(^{-1}\). They reported that two thirds of the magnesium is usually present in the serum; this is close to the figure obtained for the control in this work (62.8%).

<table>
<thead>
<tr>
<th>UHT milk sample</th>
<th>Fortification (% RDI)</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>11.4</td>
</tr>
<tr>
<td>Fortified with iron only</td>
<td>25</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>7.7</td>
</tr>
<tr>
<td>Fortified with iron, magnesium and zinc</td>
<td>25</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.3</td>
</tr>
</tbody>
</table>
3.1.3 Zinc

The average concentration of zinc in the control milk (4.59 mg.L$^{-1}$) was within the range (0.2–19 mg.L$^{-1}$) cited by Walstra et al. (2006). In the control milk, the majority (>80%) of the zinc was associated with the casein, the remainder being distributed approximately equally between the serum and fat phases (Table 4). In the zinc-fortified milks, an even higher proportion was associated with the micellar casein fraction, and this generally increased with increasing level of fortification, up to 92.7%. The distribution is similar to that reported by Flynn et al. (1989). They reported 94% associated with the casein micelle of which 63% was closely associated with the colloidal calcium phosphate and 31% was loosely associated with the caseins. Silva et al. (2001) reported that in the micelle, zinc, along with magnesium and calcium, is mostly associated with the calcium phosphate. Since most added zinc became associated with the casein micelle, it can reasonably be assumed that it associated with the calcium phosphate also (Table 4).

### Table 3  Magnesium distribution in control and mineral-fortified UHT whole milks (mean values, n=3)

<table>
<thead>
<tr>
<th>UHT milk sample</th>
<th>Fortification (% RDI)</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>62.8</td>
</tr>
<tr>
<td>Fortified with magnesium only</td>
<td>25</td>
<td>65.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>69.8</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>66.3</td>
</tr>
<tr>
<td>Fortified with iron, magnesium and zinc</td>
<td>25</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>72.4</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>77.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>62.8</td>
</tr>
</tbody>
</table>

### Table 4  Zinc distribution in control and mineral-fortified UHT whole milks (mean values, n=3)

<table>
<thead>
<tr>
<th>UHT milk sample</th>
<th>Fortification (% RDI)</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>10.2</td>
</tr>
<tr>
<td>Fortified with zinc only</td>
<td>25</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>3.6</td>
</tr>
<tr>
<td>Fortified with iron, magnesium and zinc</td>
<td>25</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5.6</td>
</tr>
</tbody>
</table>
3.2 Sensory evaluation

3.2.1 Stage 1: UHT milks fortified to 75% RDI per litre

Triangle tests based on overall sensory differences were used to determine whether fortified UHT milk samples could be differentiated from control UHT milk samples by a trained sensory panel. The results for colour and aroma (not presented) indicated no significant difference in the responses for the fortified and control samples \((P>0.05)\) in all the three sessions.

The responses for taste indicate that panellists significantly perceived differences in taste of the iron-fortified samples \((P>0.05)\) but not of the zinc-fortified samples \((P>0.05)\) compared with the control samples in all three sessions. In two of the three sessions, the panellists significantly \((P>0.05)\) identified differences in taste of the magnesium-fortified samples from the control UHT milk samples (Table 5).

Since the panel could perceive differences between the control UHT milk and the UHT milk fortified with Fe and Mg to 75% RDI, it was decided to reduce the level of fortification to 50% RDI for the storage trial in which sensory evaluation was also conducted.

No sensory evaluation was performed on samples fortified to less than 50% RDI.

3.2.2 Stage 2: UHT milks fortified to 50% RDI per litre and stored at 30 °C for 60 days

The storage trial contained an additional sample, one to which all three minerals (of iron, magnesium and zinc, each to 50% RDI) were added. The results of the duplicate triangle tests with fortified and control UHT milk samples after 7, 30 and 60 days of storage are presented in Table 5. For samples with \(P\) values <0.05, the panellists were able to differentiate between fortified and control samples. The results suggest that after 7 days of storage, the panellists could not perceive any difference in taste between control and fortified UHT milk samples. However, they could differentiate the iron-

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### Table 5  Panellists’ ability to differentiate between control UHT whole milk and UHT whole milk fortified with Mg, Zn, Fe and all (Mg, Zn and Fe) to 75 and 50% of recommended daily intake per litre

<table>
<thead>
<tr>
<th></th>
<th>Mg</th>
<th>Zn</th>
<th>Fe</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>75% of RDI per litre</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage (day 10)</td>
<td>0.00</td>
<td>0.07</td>
<td>0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Storage (day 10)</td>
<td>0.09</td>
<td>0.09</td>
<td>0.01</td>
<td>NA</td>
</tr>
<tr>
<td>Storage (day 10)</td>
<td>0.00</td>
<td>0.09</td>
<td>0.01</td>
<td>NA</td>
</tr>
<tr>
<td><strong>50% of RDI per litre</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage (day 71)</td>
<td>0.22</td>
<td>0.43</td>
<td>0.22</td>
<td>0.06</td>
</tr>
<tr>
<td>Storage (day 30)</td>
<td>0.09</td>
<td>0.59</td>
<td>0.02</td>
<td>0.61</td>
</tr>
<tr>
<td>Storage (day 60)</td>
<td>0.67</td>
<td>0.00</td>
<td>0.00</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*NA* not applicable
fortified UHT milk from the control sample after 30 days of storage and after 60 days of storage, they could differentiate both iron- and zinc-fortified UHT milk samples from the control ($P>0.05$, Table 5). The panellists could not perceive any sensory difference between the control and the samples fortified with magnesium and all three minerals.

The sensory results show the potential for as well as the limitations in fortifying UHT milk with Fe, Mg and Zn. The Fe results show that addition of ferrous sulphate causes instability in the product during storage which would limit its application. Although ferrous sulphate is soluble in milk, imparts little colour and contains iron in a bioavailable form, Fe fortification using ferric iron may be preferable for product stability. Recently, Mittal et al. (2012, 2013) reported the preparation of a soluble ferric iron–milk protein complex from calcium-depleted milk which has potential for fortification of foods including UHT milk products. The use of iron–milk protein complexes for fortifying foods has recently been reviewed by Ellis et al. (2012). The results for Mg indicate considerable potential for fortification of UHT milk, provided it is accompanied by addition of citrate to prevent fouling during processing, while the zinc results suggest only low levels could be incorporated without induction of off-flavours during storage.

3.3 Physico-chemical characteristics of UHT milk fortified to 50% RDI and stored at 30 °C for 60 days

3.3.1 Viscosity

The viscosity data are shown in Fig. 1. The viscosity was similar for all milk samples at zero time. After 60 days, there were no significant differences between the iron-, magnesium- and zinc-fortified milks, but the results showed significant differences with the control ($P>0.05$). The viscosity of all milk samples gradually increased during storage ($P>0.05$) with that of the milk samples fortified with all three minerals increased significantly ($P>0.05$) more than the viscosities of the control and samples fortified with one mineral only. However, all remained below 10 mPa.s, the level at about which gelation occurs (Kocak and Zadow 1985). There were no signs of clotting or gelation (Datta and Deeth 2007).

![Fig. 1 Viscosity during storage of UHT whole milks fortified with Fe, Mg and Zn to 50% recommended daily intake per litre (mean values, $n=3$).](image-url)
3.3.2 Lipid oxidation

All values of TBARS were below the threshold levels at which an oxidized flavour could be perceived (King 1962). They showed moderate increase during the first 40 days of storage and then a more rapid increase up to 60 days of storage. There was no significant difference between the control and zinc-fortified samples which showed the smallest increase during the storage time (Fig. 2). The greatest increase occurred in the samples containing iron. Similar results were obtained with the iron-fortified sample and the sample fortified with all three minerals. According to King (1962), the description of the flavour of milk with the highest levels of oxidation observed here would be “questionable to very slight”. Although whole milk (3.89% fat) was fortified, processed and bottled in PET transparent containers, the low levels of oxidation in the milk samples can be attributed to their being stored in the dark and having minimal headspace (Smet et al. 2009). In addition, sulphydryl compounds

![Fig. 2](image1.png)

**Fig. 2** Thiobarbituric acid reactive substances (TBARS) during storage of UHT whole milks fortified with Fe, Mg and Zn to 50% recommended daily intake per litre (mean values, \( n=3 \)). •• Control. •• Fe. •• Mg. ••• Zn. • All

![Fig. 3](image2.png)

**Fig. 3** Proteolysis during storage of UHT whole milks fortified with Fe, Mg and Zn to 50% recommended daily intake per litre (mean values, \( n=3 \)). •• Control. •• Fe. •• Mg. ••• Zn. • All
produced during UHT treatment would have acted as antioxidants (Shipe et al. 1978). Greater oxidation would be expected with a longer storage period, especially in the samples containing iron which is a well-known pro-oxidant.

### 3.3.3 Proteolysis

The level of proteolysis in all milks, including the control, increased slowly during the first 36 days and then increased at a higher rate (Fig. 3). Overall, the increases were small and there was no obvious effect of the minerals added on the proteolysis level.

### 4 Conclusion

Sensory evaluation of UHT milks fortified with various levels of iron, magnesium and zinc revealed that the trained panellists could perceive the taste of milk samples fortified to 75% of RDI per litre but not those fortified to 50%. During storage for 60 days at 30 °C, milk fortified to 50% of RDI per litre with iron and zinc exhibited off-flavours mostly due to oxidation, which was generally consistent with the TBARS results. Viscosity and proteolysis levels increased slightly during the storage period but not to a level which would cause instability in the product.

The distributions of iron, magnesium and zinc among the casein, serum and fat phases of the fortified milk samples were similar to those in unfortified milks. However, increasing fortification generally increased the proportion in the preferred phase, that is, casein micelle for iron and zinc and serum for magnesium. Neither UHT processing nor fortification changed the distribution of the minerals in milk.

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### References


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