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IMPACT OF LOW REFRIGERATION TEMPERATURE ON COLOUR OF MILK

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The purpose of this study was to investigate the effect of low refrigeration temperatures on bacterial numbers that affect the colour in fluid pasteurised skim milk. Standard plate count increased at 2 and 5 °C with increasing storage days. Bacterial numbers were within the acceptable level at 2 °C until 17 days, beyond the shelf-life of 14 days compared to 5 °C. The decrease of pH in milk was observed with increasing bacterial numbers at both temperatures. The change of colour in the milk was determined using a colorimeter. Values of *L** gradually started to change with increased storage days. The negative values of Hunter *a** provided the evidence of green and the positive values of Hunter *b** indicated the yellow colour in milk samples at the end of the storage day. Results showed increased bacterial numbers were correlated with the values of *L**, *a**, and *b** obtained from the colour measurement. This study showed that even low refrigeration temperatures do not prevent microbiological, chemical, and sensorial changes in milk during storage, however, such changes are slowed down.

Keywords: pasteurised skim milk, low temperature, bacteria, colour

Milk is one of the most essential and nutritious foods for humans, which contain water, proteins, lipids, carbohydrates, enzymes, vitamins, and minerals (Nicolaou & Goodacre, 2008). Milk supports the growth of bacteria because of its specific composition and near neutral pH ranging from 6.5 to 6.8 (Chandan et al., 2008; Anderson et al., 2011).

Storage temperature has a significant influence on the number of bacteria in milk. Microorganisms that have the ability to grow at low temperatures are mostly psychrotrophic bacteria. These bacteria can multiply at or below 7 °C, but have optimal and maximal growth temperatures above 15 and 20 °C. Bacterial spoilage occurs when psychrotrophic microbes increase in numbers and become the dominant microflora during refrigerated storage (Magan et al., 2001; Moyer & Morita, 2007), resulting in poor quality of milk.

At higher temperature milk spoils rapidly, because a rise in temperature of a few degrees can increase microbial growth (Simon & Hansen, 2001; FSANZ, 2009). Thus, milk needs to be stored at 5 °C or below across the food supply chain to achieve longer shelf-life, because low temperature can slow down the chemical changes and growth of many bacteria (Al-Qadiri et al., 2008; FSANZ, 2009). According to the FSANZ (2006), the shelf-life of pasteurised milk is 7 to 14 days if milk is kept under standard storage conditions, but seasonal and regional differences may also occur.

Consumers check ‘best before date’, which provides an estimate of the shelf-life of milk during the purchase, but milk can spoil prior to printed expiration date (Lu et al., 2013). The microbiological standard for milk varies from country to country. In New Zealand, the acceptable microbiological limit for pasteurised milk is 5×10^4 CFU ml^{-1} (FSANZ, 2006).

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Colour is one of the sensory attributes of milk, which influences the milk quality, freshness, and food safety. Milk has a natural colour because of reflectance of light by dispersed milk fat globules, proteins, and natural milk pigments such as riboflavin and carotenoids (Noziere et al., 2006; Solah et al., 2007). Bacterial growth can cause sensory deterioration of milk, such as colour, texture, odour, and taste, which is unacceptable commercially (Ahmed & Abdellatif, 2013; Samet-Bali et al., 2013). There is little information available on changes of colour at low refrigeration temperatures in pasteurised skim milk. The objective of this study was to investigate i) the microbial growth at two low refrigeration temperatures (2 and 5 °C), ii) the impact of low temperatures on the colour of milk, and iii) changes of colour of milk during storage due to bacterial activity.

1. Materials and methods

1.1. Samples

A total of fifty-three 1 litre bottle of pasteurised skim milk samples were purchased from a local supermarket during June and July. The ‘best before date’ for milk normally sold in supermarkets is calculated on the basis of 14 days shelf-life.

1.2. Preparation of diluents and media

The media for standard plate count (SPC) was prepared from Standard Method Agar (Difco, Becton, Dickinson and Company, France) and distilled water. Diluent was prepared using Bacto peptone and distilled water. In this study, visible bacterial populations were counted using SPC but not identified. The Petri dishes were incubated at 25 °C for 72 h for detection of high viable bacterial counts in refrigerated milk (Van Der Zant & Moore, 1955; Moyer & Morita, 2007). The colonies were then counted and recorded for later analysis and interpretation.

1.3. Determination of pH

The pH values of milk were measured using a pH meter (Hanna, Fisher Scientific Company, Pittsburgh, PA) to detect changes in the quality of milk throughout the shelf-life.

1.4. Colour analysis

The colour of milk was determined at room temperature using a D-25 Hunterlab Miniscan XE Plus colorimeter (Hunter Associates Laboratory Inc., Reston, Virginia, USA). Colour values in Hunter Lab were evaluated in terms of L^*, which ranges from zero (black) to 100 (white), a^* represents the axis from red (range+60) to green (range–60), and b^* represented the axis from yellow (range+60) to blue (range–60). The instrument was placed over the sample container and colour values were recorded five times (n=5) for each milk sample. As combining a^* and b^* provides a better indication of colour than their individual values, hue angle was calculated by using the equation: $h° = \tan^{-1}(b^*/a^*)$. Chroma determining the degree of difference of hue was calculated using the equation: $C^* = \sqrt{(a^{*2}+b^{*2})}$. In order to compare the total colour differences (ΔE^*) between the colour properties of milk samples and those obtained after the milk was stored at two different temperatures, the following equation was used: $\Delta E = \sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$.

1.5. Statistical analysis

Analysis of variance for total bacterial counts, pH, and colour was carried out using the Excel 2007 software programme (Microsoft, CA, USA). Data were also subjected to Student's *t*-tests. The statistical significance was determined at $P<0.05$.

2. Results and discussion

2.1. Determination of bacterial numbers in milk at two low refrigeration temperatures

Total bacterial numbers in skim milk samples and the pH are shown in Figure 1.

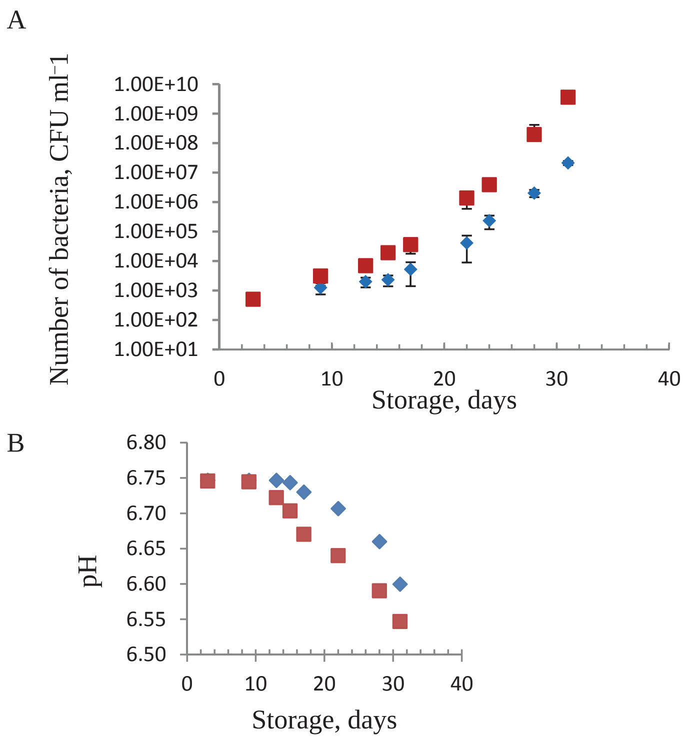

Fig. 1. Changes in total bacterial numbers (A) and the effect of pH (B) in pasteurised skim milk versus storage days at refrigeration temperatures of 2 °C (◆) and 5 °C (■)

On collection day, fresh milk samples were tested for total bacterial numbers and low SPC ($<1\times10^3$ CFU ml^{-1}) was observed. Bacterial counts remained within the acceptable numbers (1×10^4 CFU ml^{-1}) until 14 days at these storage temperatures (FSANZ, 2006). Results of the statistical analyses showed that the differences of bacterial numbers were not significant ($P>0.05$) until 14 days of storage for milk samples stored at 2 and 5 ºC. Milk samples stored at 2 °C continued to show low bacterial numbers and were within the acceptable level until day 17 compared to 5 °C. However, after 17 days, bacterial numbers in milk stored at 5 °C exceeded the acceptable microbiological level compared to the milk samples stored at 2 °C. It was observed that at 5 °C bacterial numbers continued to grow rapidly after 22 days (>1×10^6 CFU ml^{-1}) and reached >1×10^9 CFU ml^{-1} after 31 days of storage. In contrast, for milk stored at 2 °C, the numbers of bacteria reached 1×10^6 CFU ml^{-1} on day 28, and after 31 days the bacterial numbers were still lower (>1×10^7 CFU ml^{-1}) compared to 5 °C.

Overall, the results show that the prolonged storage of skim milk at low refrigeration temperatures of 2 to 5 °C can significantly change microbial population, which closely agrees with the findings of SAMARZIJA and co-workers (2012). According to some studies, the organoleptic changes in milk become detectable when bacterial counts are 1×10^6 CFU ml^{-1} or higher (BISHOP & WHITE, 1986; CLARK et al., 2009), which is considered the end of the shelf-life of pasteurised milk (RAVANIS & LEWIS, 1995). NICHOLAS and ANDERSON (1942) found that the shelf-life of pasteurised milk could be two weeks or more before spoilage would occur if milk is stored at 4.4 °C. GRIFFITHS and co-workers (1988) and BURDOVA and co-workers (2002) reported that lowering the storage temperature from 6 to 2 °C can increase the shelf-life of milk by 75%, and if the temperature is decreased by about 1 °C in the range from 5 °C to 0 °C, it suppresses the growth of microorganisms better than that of reducing temperature by 1 °C at higher temperatures.

The average pH of fresh milk samples was 6.75. Statistically, no significant difference (P>0.05) was observed between these two storage temperatures until 14 days. The pH value of milk remained within the normal ranges from 6.60 to 6.75 at these temperatures until 21 days. However, the samples showed a decrease in pH with increase in storage days at both temperatures, and consequently bacterial numbers were also increased.

2.2. Characterisation of colour attributes of milk

2.2.1. Analysis of lightness (L*) *values and their relationship with bacterial numbers. L** values of milk samples stored at 2 and 5 °C over the period of 31 days of storage and the relationship between *L** and bacterial numbers is shown in Figure 2.

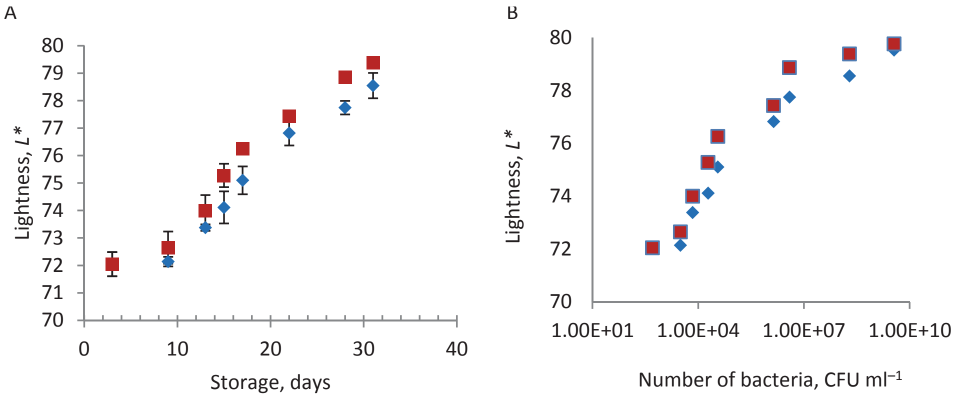

Fig. 2. Effect of storage days on changes of lightness (A) and the relationship between bacterial numbers and lightness (B) in pasteurised skim milk at 2 °C (◆) and 5 °C (■)

On day 1, the average Hunter *L* value of fresh milk samples was 72.046. After 8 days, the *L** values gradually started to change with increasing storage time. *L** values were 75.276 at 5 °C and 74.112 at 2 °C after 14 days. The differences between the samples at two storage temperatures were found to be significant (P<0.05) over 31 days. Increasing *L** values at 2 and 5 °C indicated that milk had relatively light colour at the end of storage. According to Kneifel and co-workers (1992), skimmed milk has lower *L** value than full-fat milk. The characteristics of the white colour of milk may be due to scattering of light by the contained colloidal particles, for example, milk fat globules, calcium phosphates, and casein micelles (Belitz & Grosch, 1999; Chandan et al., 2008).

It was also investigated whether the changes in bacterial numbers have an influence on parameter *L** at these two low temperatures. The regression analysis of the data indicated a linear relationship with a correlation coefficient of 0.888 at 5 °C and 0.926 at 2 °C. The least squared regression lines were: y=0.53 ln x+69.70 at 5 °C and y=0.73 ln x+68.09 at 2 °C. These equations predict that the increasing bacterial numbers have an impact on *L** of milk at both 2 and 5 °C. In refrigerated pasteurised milk, many microorganisms thrive when the pH of the milk changes. Higher bacterial growth in milk results in rapid use of oxygen by bacteria, which turns milk white (Babcock & Parker, 1932).

2.2.2. Analysis of greenness (a*) *values and its relationship with bacterial numbers.* Analysis of greenness *(a*)* values with extended storage period and its relationship with bacterial numbers are shown in Figure 3.

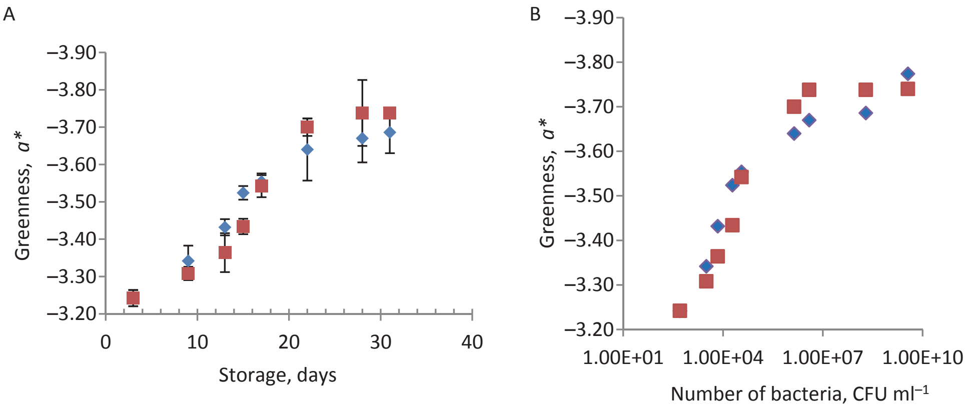

Fig. 3. Effect of storage days on changes of greenness (A) and the relationship between bacterial numbers and greenness (B) in pasteurised skim milk at 2 °C (◆) and 5 °C (■)

On day 1, the Hunter a value of fresh milk was –3.242. On the basis of results obtained, the differences of *a** values for the two storage temperatures were not significant (P>0.05) until 9 days. However, after 14 days the values slightly changed to –3.434 at 5 °C and to –3.524 at 2 °C. Statistical analysis showed that at this prolonged storage period the colour of milk changed toward greenness significantly ($P<0.05$). A green colour in milk is appeared to be influenced by riboflavin, which is a strong photosensitizer. Riboflavin can absorb visible light, converting this energy into oxygen, which is highly reactive (Chandan et al., 2008). It was observed from the result that the *a** values changed with increasing bacterial numbers. Regression analysis of the data indicated a linear relationship with a correlation coefficient of 0.822 at 5 °C and 0.802 at 2 °C. The least squared regression lines were: y=0.04 ln x–3.10 at 2 °C and y=0.03 ln x–3.09 at 5 °C.

2.2.3. Analysis of yellowness (b*) *in milk and its relationship with bacterial numbers.* The parameter *b**, which measures the differences in the yellow and blue colour components showed an increase in *b** value (towards yellow) after 31 days of storage (Fig. 4).

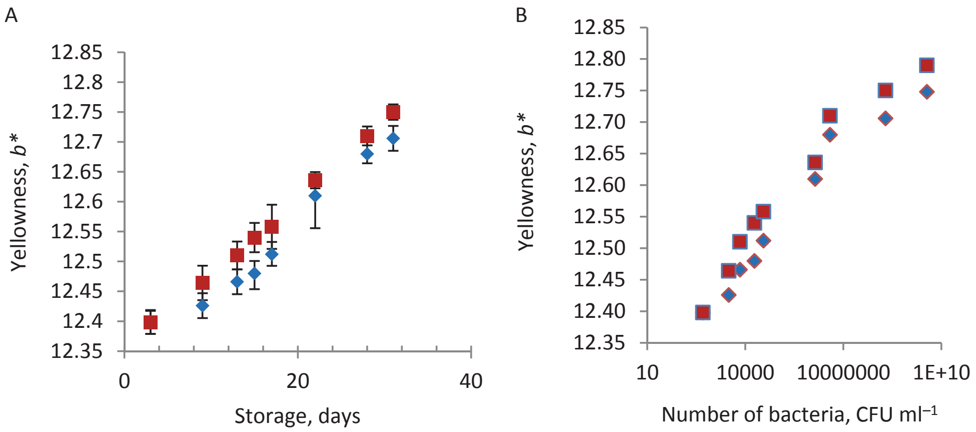

Fig. 4. Effect of storage days on changes of yellowness (A) and the relationship between bacterial numbers and yellowness (B) in pasteurised skim milk at 2 °C (◆) and 5 °C (■)

The Hunter *b** for fresh milk samples on day 1 was 12.298. The *b** at both temperatures showed a linear increase. The results showed that the values did not significantly vary between these two low storage temperatures. This study found that the changes in bacterial numbers have an influence on yellowness (*b**) of milk at the two storage temperatures. Regression analysis of the data showed a nearly linear relationship with a correlation coefficient of 0.957 at 5 °C and 0.951 at 2 °C. The least squared regression lines were: y=0.025 ln x+12.28 at 5 °C and y=0.034 ln x+12.20 at 2 °C. The yellow colour in milk may be a direct result of the growth of certain microorganisms (Hammer, 1915; Tauro et al., 1986).

2.2.4. Comparison of Hunter's colour attributes in pasteurised skim milk samples. Differences in the colour were found in milk samples stored at refrigeration temperatures of 2 and 5 °C. The Hunter-*L*, *a*, *b* components of pasteurised skim milk samples on day 1 and day 31 are shown in Table 1.

Table 1. Comparison of Hunter colour attributes of fresh and stored milk

Samples	L^*	a^*	b^*	C^*	$h°$	ΔE
Fresh milk	72.0	–3.24	12.3			
2 °C	79.5	–3.74	12.7	13.2	34	7.50
5 °C	79.8	–3.77	12.8	13.3	39	7.74

L^*: Lightness; a^*: greenness; b^*: yellowness; C^*: chroma; $h°$: hue; ΔE: total colour differences; Fresh milk: milk samples on day 1; 2 °C and 5 °C: milk samples on day 31

The high values of L^* indicated that the milk samples were light. The analysis of variance identified no significant (P>0.05) differences in L^* values between the milk samples stored at 2 °C and 5 °C. Kneifel and co-workers (1992) reported the L^*, a^*, and b^* values (L^*=81.7, a^*= –4.8, and b^*=4.1) for pasteurised milk (<0.1% fat content) using CIELab. They compared their values with Giangiacomo and Messina (1988) (L^*=88.2, a^*= –4.35, and b^*=5.40) and Bosset and Blanc (1978) (L=95.5, a= –2.0, and b=12.6), who used CIELab and Hunter's systems, respectively. Hunter's colour values found for pasteurised skim milk in this study were partly different from these values. The differences in colour values may occur due to different colour analysis systems, origin, and fat content of milk samples.

The chroma (C^*) value indicated the colour intensity of milk samples, the higher the C^* value, the higher the colour intensity of samples was. While comparing C^* values at these temperatures, no significant changes in colour intensity were found. The $h°$ value in milk is related to the differences in absorbance at different wavelengths of light. Considering the qualitative attributes of colour, the $h°$ in this study showed that milk stored at 5 °C was slightly more intense in colour than milk at 2 °C.

3. Conclusions

The study found that the growth of bacteria was more rapid at 5 °C than 2 °C. The prolonged period of storage and the apparent increase of bacterial activity resulted in changes of colour in milk stored at low temperatures. Low storage temperatures do not prevent microbiological, chemical, and sensorial changes in milk, however, those changes are slowed down. Milk deteriorates during an extended period of storage regardless of the low refrigeration temperature.

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