The Potential of Conductivity, Redox Potential and Dissolved Oxygen in Raw Milk Quality Prediction

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Abstract*: Changes in milk quality are associated with changes in dissolved oxygen (DO), redox potential (E_h) and conductivity (Co) reflecting the potential of these parameters in quality prediction. However, limited interpretation of those changes/results limits the application of the same in raw milk quality prediction. The aim of this study was therefore to explore the potential of DO, E_h and Co in milk quality assessment. Raw milk, lactoperoxidase system (LP-s) activated raw milk and LP-s activated Ultra High Temperature (UHT) milk inoculated singly with pure strains were used in this study. The performances of DO, E_h and Co in quality prediction were assessed against the objective methods: pH, titratable acidity (TA), alcohol stability test (AST), clot on boiling (COB), dye reduction tests and total viable counts (TVC). The results showed that any negative E_h value, a Co value greater than the initial Co value and DO around zero or below detection limit all indicated spoilage. These corresponded well with increases in microbial numbers indicative of spoilage recorded at about 7 log cycles of bacterial counts. Co and E_h were in consistent agreement in quality estimate with AST and TA but more sensitive than COB and pH. On the other hand, DO showed similar sensitivity as COB and pH but was more lenient than AST and TA. The results generally confirmed the suitability of DO and E_h for routine analysis in both normal and LP-s activated milk; however, the use of Co requires a prior knowledge of the initial Co value of milk under test which complicates its use in routine analysis.*

Key words: Quality prediction, conductivity, redox potential, dissolved oxygen

INTRODUCTION

Testing procedures are a crucial part of quality control process to comply with approved standards and meet the requirements for chemical composition, purity and microbial levels. Microbial quality is a very important aspect; however, standard plate count tests for determining total microbial count require incubation periods of three days at 30°C, so there is considerable interest in simple rapid methods which would be suitable in developing countries. Physical methods are easy to apply and so provide good supplementary tests to the lactoperoxidase system (LP-s). However, for any method to be precisely applied sufficient interpretation of the results should be available. This is the main limitation in the use of electrochemical properties of milk such as dissolved oxygen (DO), conductivity (Co) and redox potential (E_h) in quality assessment apart from their great potential. The aim of this study was therefore to exploit the changes in DO, Co and E_h in quality assessment, and specifically:

- to assess the performances of DO, E_h and Co against the objective methods: pH, TA, AST, COB, dye reduction tests and TVC;
- to establish the discrimination criteria of milk quality based on DO, E_h and Co;
- to establish the relationship between changes in Co, DO and E_h and the increase in bacterial counts;
- to test the sensitivity of the methods in following variations in keeping quality (KQ) among milk samples preserved by different LP-s.

The results would then be interpreted with the purpose of establishing the base for milk acceptance and/or rejection.

MATERIALS AND METHOD

Materials

Basic Experiments in the UK

Sodium percarbonate ($Na_2CO_3.1.5H_2O_2$) was used as a source of H_2O_2 for LP system activation; thiocyanate (SCN^-) was in the form of NaSCN and I^- in the form of KI (all supplied Sigma Aldrich, Gillingham, UK). Bovine LP was kindly donated by DMV International (New York, NY). Raw bovine milk was collected from the Reading University farm and homogenized, full-cream UHT milk was supplied by Arla Foods, (Leeds, UK).

Field Trials in Tanzania

SCN^- was in the form of NaSCN (from BDH chemicals Ltd., Poole, UK), I^- in the form of KI (Fisher scientific, Leicestershire, UK) and Sodium carbonate peroxyhydrate was used as a source of H_2O_2 for LP system activation (Peroxide-chemie, GmbH, Munich). Bovine LP was kindly donated by DMV International (New York, NY).

Raw milk was collected from commercial farms and individual farmers in Morogoro Municipality.

Test Organisms and Growth conditions

Three strains, *Staph. Aureus* (NCDO 10651), *P. aeruginosa* (NCTC 10651) and *Bacillus cereus* (NCDO 0577) obtained from the stock culture of the Department of Food Biosciences, Reading University maintained at -80°C in cryoprotective media, were used in this study. The culture were selected on the basis that they have the potential to cause spoilage in raw milk and pasteurized products. Cultures were grown as described by Fweja *et al*. (2008). They were regenerated in nutrient broth and grown overnight on nutrient agar slants at 30°C. Single colonies were isolated from stock slants by streaking on to agar plates at 30°C for 24h, and purity of the test organisms was confirmed by microscopic examination. Working inocula for antibacterial assays were prepared by sub-culturing single colonies from agar plate streaks into nutrient broth and incubating at the optimum growth temperatures of the organisms: 25°C (*P. aeruginosa*), 37°C (*Staph. Aureus*), and 30°C (*B. cereus*) for 18 to 20 hours. The number of viable cells was around 10^7 to 10^8 cfu/mL of broth.

Preparation of the LP+GE+E System substrate
Garlic was skinned and the central part ground in a sterile mortar. The resulting concentrated extract was filtered through muslin cloth and then through Whatman filter paper no. 4. The concentrated garlic extract was diluted (1% vol/vol) in distilled water. Each 1 mL of 1% garlic extract combined with 1% ethanol was considered to be equivalent to 10 mg/kg as claimed previously by Jandal (1998).

Activation of the LP-s in raw milk (on field trials)
This was done according to Fweja *et al.* (2007) where activation of the LP-s was achieved by addition of sodium thiocyanate (BDH chemicals Ltd., Poole, UK) or potassium iodide (Fisher scientific, Leicestershire, UK) followed by sodium carbonate peroxyhydrate (Peroxide-chemie, GmbH, Munich). The concentrations of activators quoted refer to the calculated concentrations of SCN^-, I^- or H_2O_2 added to the milk.

Three treatment strategies based on SCN- were used with varying concentrations of the activators. The treatments involved were (i) equal concentrations of SCN^- / H_2O_2 i.e. 7:7, 10:10 and 15:15 mg/l, (ii) excess concentrations of SCN^- in the SCN^- / H_2O_2 combination i.e. 15:10, 20:10, and 25:10 mg/l and (iii) excess concentrations of H_2O_2 in the SCN^- / H_2O_2 combination i.e. 10:15, 10:20 and 10:25 mg/l.

The treated and control milk samples were dispensed in 20 ml glass bottles (sterilized by oven drying at 100°C and stored overnight (for approximately 10 h) at room temperature which varied from 28-30°C. The final design of the experiment was determined from preliminary trials (results not shown) which showed that fresh raw milk could keep for up to 10 h in tropical condition (during field trials). Sample assay was carried out at 0 h and 10 h and then after every 2 h to an end point as determined by the quality prediction methods.

Activation of the LP-s of UHT milk inoculated singly with pure strains
The concentration of LP used was 30 mg/kg based on its natural concentration in milk (De Wit and Van Hooydonk, 1996). The H_2O_2 concentration of 80 mg/kg was adopted in this experiment because the findings of Ewais *et al*. (1985) demonstrated effective performance of the LP + SCN^- + H_2O_2 system of 70 to 80 mg/kg, ensuring an extended shelf life of 24 h. The recommended SCN level is between 3.5 and 12 mg/kg (Rossi and De Oliveira, 1993-94) or ≤ 10 mg/kg (Ewais *et al*., 1985). Therefore the concentration of 7:80 mg/kg of SCN^-: H_2O_2 was chosen to activate the LP system in these experiments. For comparison the same substrate level was adopted for LP + I^-+ H_2O_2 system; that is, 7:80 mg/kg of I^-: H_2O_2. Substrates for the LP + GE + E were similarly established based on earlier work (Jandal, 1998) and preliminary trials in raw milk (data not shown) which gave the best KQ for 110:30 mg/kg garlic extract: ethanol combination level. The composition for the reaction mixtures for the different LP-s were as follows: LP + SCN^-+ H_2O_2 (30:7:80 mg/kg), LP + I^- + H_2O_2 (30:7:80 mg/kg) and LP + GE + E (30:110:30 mg/kg).

Lactoperoxidase, thiocynate, iodide, and sodium percarbonate were dissolved separately in 1 mL of HPLC water and subsequently added to whole UHT milk (inoculated with test strains) individually through syringe filters.

Determination of KQ
KQ was determined in duplicate samples using the test methods i.e. DO, Co and E_h alongside the objective methods, which included, pH, titratable acidity (TA), alcohol stability test (AST), clot on boiling test (COB), rezazurin test and methylene blue.
Test Methods for Determining KQ

Dissolved Oxygen (DO)
DO was measured using MX300 Meter (Mettler-Toledo GmbH, Analytical, CH-8603 Schwerzenbach, Swirtzerland) connected to InLab 681 DO Sensor Module and results expressed as a percentage of saturation. The instrument was calibrated daily (according to the manufacture's instructions) by exposing the sensor in air until when a 100% value is displayed. The sensor was then inserted into the milk sample to the desired depth and agitated to dislodge air bubbles from the sensing area and the reading recorded when the stability indicator appeared. Agitation of the probe is recommended to reduce errors caused by consumption of small amounts of O_2 by the probe which can cause lowering of the O_2 concentration in the boundary layer between the sample and the probe membrane.

Redox Potential (E_h)
MX300 meter connected to the InLab 581 Redox Sensor Module was used in measuring the E_h (mV). The meter was first checked against a standard solution with $E_h = 468 \pm 5\ mV$ prior to undertaking any sample measurement; the reading was recorded when stable.

Conductivity (Co)
Conductivity was measured using an MX300 meter connected to InLab 781 Co Sensor Module and the results expressed as millisiemens / cm (mS / cm). A two point calibration in air and in 0.1 M KCl solution (12.88 mS/Cm) was performed. The sensor was placed into the milk sample such that the cell chamber slot was completely immersed in the sample with an auto end point set. The reading was recorded when a stable end point was reached.

Objective methods for Determining KQ
For titratable acidity (TA) 1 ml of phenolphthalein solution (0.5% w/w) was added to 10 ml of milk. The mixture was titrated against 0.111 *M* NaOH to an end point marked by a pink colour persistent for at least 5 seconds. The milk was judged to be spoiled when TA had increased by 0.02 units (Chakraborty *et al.,* 1986).
The pH was determined with a pH meter (ABB Kent EIL 7045/46, Kent, England) and spoilage was considered to have occurred by a decrease in pH of 0.4 units.
Clot on boiling (COB) was performed by boiling 2 ml of milk sample in a test tube for 5 minutes after which it was examined for curdling by tilting the tube gently. The sample was judged to be spoiled when clotting was observed (Harding, 1995).

For the alcohol stability test (AST), equal volumes of milk and 70% ethanol (prepared from 99% ethanol, VWR International Ltd.) were mixed in a test tube. The tube was inverted several times and then examined for coagulation. Coagulation of milk indicated spoilage (Marks *et al*., 2001).

Methylene blue (MB) was performed by mixing 10 ml of milk with 1 ml of MB (0.05%) and incubated in the water bath set at 37°C and examined after every 30 min for decolouration. Samples which decolourised in < 2 h were considered to have spoiled (O'Connor, 1994).

For Rezazurin 10 ml milk was mixed with 1 ml of 0.005% rezazurin and incubated in the water bath at 37°C. After 10 min, a sample was taken out and contents transferred into Lovibond tube and then slotted into the Lovibond comparator alongside the control sample, milk without rezazurin. The disc number < 4 indicated unsatisfactory milk quality (O'Connor, 1994).

Initial Values for DO, Co and E_h

Prior to the preliminary trials on KQ initial values for DO, Co and E_h were determined. Raw milk from individual Friesian cows and the corresponding bulk milk were used. The determinations were done in duplicate samples and the experiments were repeated after two weeks.

Preliminary Trials on K.Q

For preliminary trials on K.Q bulk milk was dispensed into sterile screw capped plastic bottles, each containing 25 ml, and incubated at 5°C to provide enough time to follow the characteristic spoilage and accompanying changes in the test parameters occurring over time and at 30°C to simulate tropical conditions. This was carried out to gain a better experience of the functionality and limitations of DO, Co and E_h probes in quality prediction. Measurements were taken before and after incubation of duplicate samples for a period of 1-15 days for samples at 5°C and after every three hours for samples at 30°C. Measurements for DO, Co and E_h were carried alongside the control methods, pH, TA, COB and AST.

Keeping Quality Studies

KQ for raw milk, LP-s activated UHT milk inoculated singly with pure strains and LP-s activated raw milk was measured for the basic Lab experiments and also during field trials carried out in Tanzania. TA, pH, COB, AST tests and in some cases dye reduction methods (MB & RZ tests) were used alongside the test methods. The KQ experiments were designed and carried out using one sensor at a time to avoid the possibility of depolarisation of the sensor (as experienced in preliminary studies) and the need for recalibration during analysis of each batch of samples. For all KQ measurements, duplicate readings were taken and the reported values are means

Trials in Raw Milk

The KQ measurements for raw milk were done at 0 h, 12 h and then after every 2 h to allow enough time for bacterial multiplication. This design of experiment was adopted following preliminary trials which showed that fresh raw milk could stay overnight before developing noticeable spoilage.

Trials in LP-s Activated UHT Milk Inoculated Singly with Pure Strains

This experiment was carried out to establish the relationship between changes in Co, DO and E_h and the increase in bacterial counts. It also aimed to test the sensitivity of the methods in following variations in KQ among milk samples preserved by

different LP-s. Whole UHT milk inoculated singly with one of the three pure cultures of *S. aureus, B. cereus and P. aeruginosa* to a final concentration of approximately 10^2 cfu/ml was activated with LP/SCN^-/H_2O_2, LP/GE/E, or LP/I^- /H_2O_2 and the enumeration of TVC was performed every 5 h alongside DO or Co or E_h measurements.

Trials in LP-s Activated Raw Milk (On Field Application)

To assess the performance of the test methods in LP activated raw milk, DO, Co and E_h were also used during field trials on LP-s activation conducted in Tanzania for KQ estimation. Three treatment strategies were used based on the concentration of the activators as described earlier each with three levels of activation. Because of the close similarities in KQ estimates of LP-s activated raw milk for the test methods and the control methods at different activation levels, only the KQ estimates for the control sample and LP-s activated raw milk at one of the activation levels of each of the particular activation strategy are presented in this paper for the sake of demonstrating their performances. Measurements for KQ were determined at 0 h and 10 h and subsequently every 2 h, following the results of the initial experiments. All measurements were done at room temperature, 28 -30ºC.

RESULTS

Initial Values of DO, Co and E_h in Bulk and Individual Cows' Milk

Measurements of DO, Co and E_h were performed on 37 individual cows' and on 2 batches of bulk milk to establish the initial values of these parameters in fresh raw milk (Table 1). The E_h varied widely between individual cows' milk and also between bulk milk. However, there was a close similarity in the mean E_h of bulk milk and individual cows' milk. Wide variations were also observed in DO between individual cows' milk, however the variations were small between bulk milk. Results for Co demonstrated a narrow variation between individual cow's milk and between bulk milk and also between the two groups.

Table 1: Initial Values of Dissolved Oxygen (DO), Conductivity (Co) and Redox Potential; (E_h) of Individual Cows' (Friesian, n=37) and the Corresponding Bulk Milk (n = 2)

	DO (%)		Co (mS/cm)		E_h (mV)	
Milk samples	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Individual	46 - 73	55 ± 5.8	4.13 - 5.18	4.63 ± 0.27	53 - 300	91± 59.9
Bulk	61 - 70	67 ± 3.9	4.25 - 4.64	4.53 ± 0.15	52 - 154	97 ± 32.4

Preliminary Trials on KQ

The E_h of refrigerated samples (Table 2) increased steadily from 0 to 7 days and then decreased steadily up to 14 days with a major drop between days 14 and 15. The rapid drop in E_h to a negative value on day 15 coincided with increased TA (> + 0.02) and precipitation in ethanol i.e. AST (+). The milk however, failed to clot on boiling (COB) and the pH drop was < 0.4 units, which both suggest satisfactory quality. A similar but much accelerated pattern of changes was recorded for samples at 30°C (Table 3; Figure 1). The spoilage marked with a negative shift in E_h on 9 h, similarly coincided with the attainment of > 0.02 increase in TA and precipitation in AST. However, pH and COB were not sensitive enough at this stage to confirm spoilage.

Changes in DO for samples incubated at 5°C (Table 2) showed no clear pattern; and was characterised by the rise and fall in its content during storage. The changes in DO over 14 days incubation did not show any clear difference for quality discrimination among samples while that on day 15 could not be determined due to malfunctioning of the DO sensor. As a result, it was not possible to assess its performance against the objective methods (TA and AST), which indicated milk spoilage on day 15. Additional experiments conducted at 30°C (Table 3; Figure 1) demonstrated a consistent decline in DO with increasing milk age to 3.1% at 24 h. Coincident with the decrease in DO concentration to 3.1% was a measurable decrease in pH (> 0.4) marking spoilage and the instability of milk to COB. AST and TA showed early spoilage at 9 h, but DO could not be determined due to depolarisation of the sensor and calibration problems.

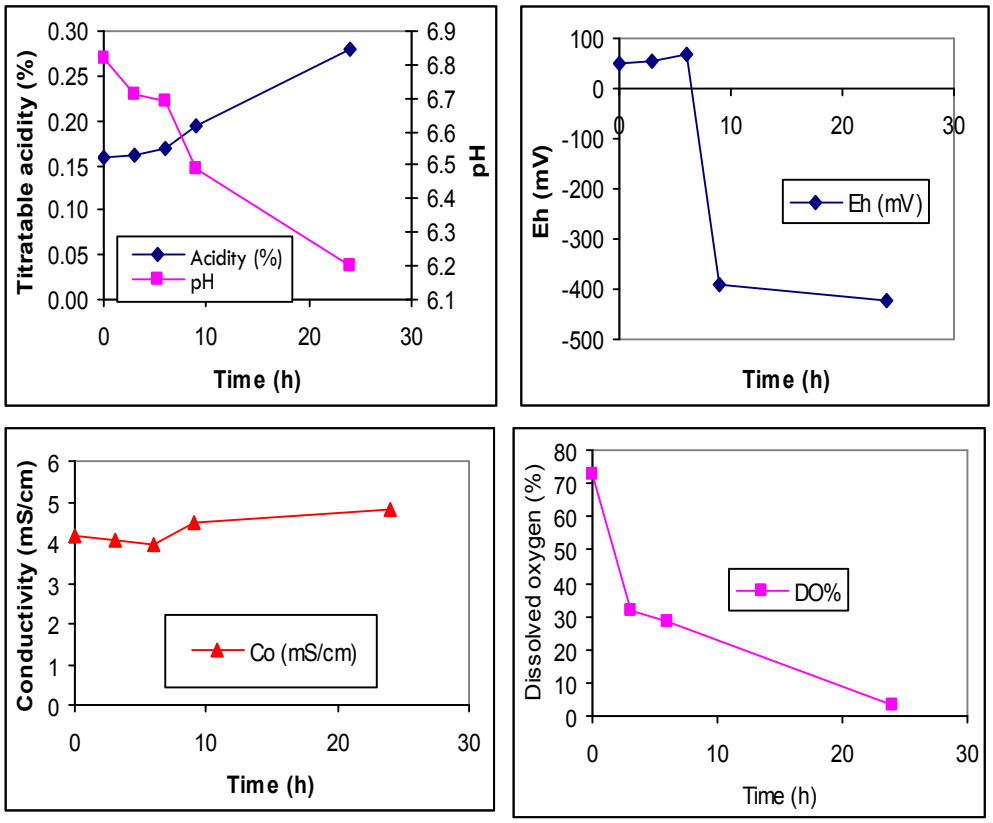

Figure 1: Changes in Dissolved Oxygen, Conductivity, Redox Potential, pH, and Acidity of Raw Milk Incubated at 30°C

Under refrigerated conditions (Table 2), Co decreased from an initial value of 4.67 mS/Cm to 2.8 mS/cm (day 14) and then rose again to 4.8 mS/Cm on day 15. The rise in Co was attributed to milk spoilage as depicted by precipitation in AST and a greater than 0.02 unit increase in TA on day 15. However, the milk was still of acceptable quality, according to pH and COB tests. A similar pattern of results was observed for samples incubated at 30°C (Table 3; Figure 1).

Table 2: Redox potential (E_h) Conductivity (Co) and Dissolved oxygen (DO) against pH, Titratable acidity (TA), Alcohol Stability Test (AST), and Clot-on-boiling Test (COB) in Raw Milk Stored at 5°C.

Time (days)	Acidity (%)	pH	DO (%)	E_h (mV)	Co (mS/cm)	AST	COB
0	0.160	6.88	72.8	37.1	4.67	(-)	(-)
1	0.165	6.88	61.3	61.7	4.47	(-)	(-)
2	0.165	6.79	34.6	80.9	4.31	(-)	(-)
3	0.166	6.82	18.1	91.7	4.37	(-)	(-)
6	0.164	6.83	42.9	116.8	4.19	(-)	(-)
7	0.167	6.82	33.9	177.8	4.31	(-)	(-)
8	0.166	6.79	33.4	128.7	4.29	(-)	(-)
9	0.164	6.74	43.3	172.5	4.04	(-)	(-)
10	0.164	6.76	75.4	151.3	3.91	(-)	(-)
11	0.164	6.73	66.2	134.1	3.86	(-)	(-)
12	0.165	6.71	62.6	124.2	3.89	(-)	(-)
13	0.174	6.67	56.0	37.4	3.90	(-)	(-)
14	0.184	6.66	45.2	34.9	2.80	(-)	(-)
15	0.193	6.55	nd*	-365.4	4.81	(+)	(-)

*nd = not determined

Table 3: Redox potential (E_h) Conductivity (Co) and Dissolved oxygen (DO) against pH, Titratable acidity (TA), alcohol stability test (AST), and clot on boiling test (COB) in raw milk (3 days old) incubated at 30°C.

Time (h)	Acidity (%)	pH	RP (mV)	Co (mS/cm)	DO%	AST	(COB)
0	0.159	6.82	50.0	4.15	72.8	(-)	(-)
3	0.162	6.71	53.6	4.06	31.5	(-)	(-)
6	0.170	6.69	70.3	3.94	28.1	(-)	(-)
9	0.193	6.49	-393.1	4.47	nd*	(+)	(-)
24	0.279	6.20	-423.1	4.80	3.1	(+)	(+)

**nd = not determined*

As is well established, a similar conductivity pattern was observed in the neutralisation reaction i.e. titration of 10 ml of 0.1 M NaOH with 0.1 M HCl (Figure .2a) and titration of 500 ml raw milk with 0.1M HCl (Figure 2b) except that, in milk, the changes in Co were so gradual and small. It was also realised that the very low Co value recorded on day 14 (2.8 mS/Cm) was partly due to the depolarization of the Co sensor following its detachment from the meter for a prolonged time. A single meter was used for all three probes, as mentioned earlier. These interruptions of changing probes were avoided in the subsequent experiments by using only one probe at a time. To further examine the characteristic change in Co, a separate experiment was carried out to follow the changes in Co when the metabolic breakdown of milk components has been initiated. To shorten the time and amplify the changes in Co, a starter culture was added to milk and samples incubated at 40°C. The Co increased from 3.54 to 4.37 mS/cm sharply after just 30 min of incubation (Table 4) and then increased steadily thereafter to 5.08 mS /cm.

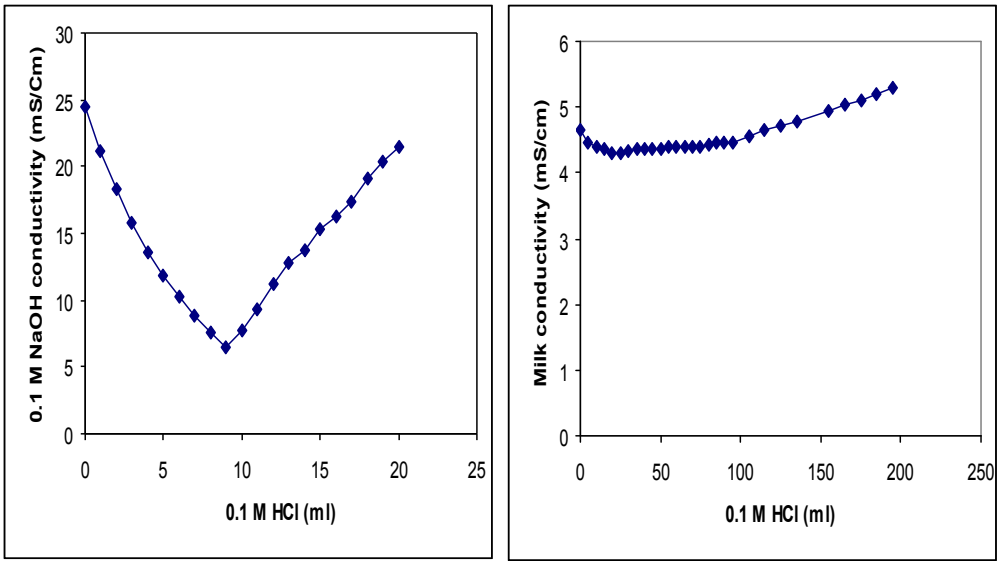

Figure 2: Changes in conductivity during titration of (a) 10 ml of 0.1 M NaOH with 0.1 M HCl and (b) 500 ml of raw fresh milk with 0.1 M HCl

Table 4: Conductivity (Co) against pH, Titratable acidity (TA), alcohol stability test (AST) and clot on boiling test (COB) in raw milk enriched with starter culture and incubated at 40°C.

Time (h)	Acidity (%)	pH	Co (mS/cm)	AST	COB
0	0.160	6.66	3.54	(-)	(-)
0.5	0.189	6.61	4.37	(+)	(-)
1.5	0.190	6.58	4.56	(+)	(+)
2.5	0.120	6.48	4.72	(+)	(+)
3.5	0.216	6.32	4.77	(+)	(+)
4.5	0.250	6.27	4.87	(+)	(+)
5.5	0.255	6.27	4.90	(+)	(+)
6.5	0.269	6.22	5.08	(+)	(+)

Trials in Raw Milk

Results for E_h and the control methods (Table 5) show a marked decrease in E_h between 12 and 14 h to a negative E_h value. This drop in E_h coincided with a > 0.02 increase in TA, alcohol precipitation of milk and a disk reading of < 4 in the 10 min rezazurin test, all of which indicating spoilage. The milk was however heat stable on boiling, the pH drop was < 0.4 units and the reduction time in case of methylene blue was > 2 h which reflects good quality. Quality estimated by E_h shows consistent agreement with that for Rezazurin, TA and AST. E_h was however more sensitive than methylene blue reduction test, COB and pH tests.

Table 5: Redox potential (E_h) against pH, titratable acidity (TA), alcohol stability test (AST), clot on boiling test (COB), Rezazurin and methylene blue reduction tests (MB) in raw milk incubated at 30°C.

Time (h)	E_h (mV)	pH	TA (%)	AST (70%)	COB	Rezazurin (10min)	M/Blue Red. Time (h)
0	73	6.73	0.160	(-)	(-)	6	>8
12	62	6.67	0.180	(-)	(-)	4	3.10
14	-425	6.50	0.197	(+)	(-)	3	2.20
16	-461	6.33	0.213	(+)	(-)	2	1.30
18	-408	6.21	0.230	(+)	(+)	2	0.30

The relationship between DO and the control methods in raw milk quality prediction (Table 6) demonstrates a decrease in DO over time to below detection limit at the end of the experiment. At this DO value, most of the objective methods had recorded unsatisfactory milk quality. The results suggest that, the drop in DO to around zero or below detection limit indicates spoilage. On this basis, TA, AST, 10 min rezazurin and methylene blue tests indicated earlier spoilage than was established by DO. The DO below detection limit however, coincided with the instability of milk on boiling, COB.

Table 6: Dissolved oxygen (DO) against pH, Titratable Acidity (TA), Alcohol Stability Test (AST), Clot on Boiling Test (COB), Rezazurin and Methylene Blue Reduction Tests (MB) in Raw Milk Incubated at 30°C. (n/d = not detected)

Time (h)	DO%	pH	TA (%)	AST (70%)	COB	Rez (10min)	MB (R/Time, h)
0	73	6.71	0.160	(-)	(-)	6	>8
12	49	6.66	0.180	(-)	(-)	4	3.20
14	34	6.52	0.197	(+)	(-)	4	2.23
16	35	6.31	0.223	(+)	(-)	2	1.27
18	n/d	6.23	0.240	(+)	(+)	2	0.40

A similar trend of changes in Co observed in earlier experiments was also found in this experiment. Prior to spoilage, there was a slight decrease in Co followed by a consistent increase (Table 7). The increase in Co above the initial Co value was recorded after 14 h which signifies spoilage as demonstrated earlier. This corresponded with a positive response to AST and a > 0.02 units increase in TA, a disk reading of 3 and < 2 h reduction time for methylene blue test; all of which indicate spoilage. However, pH and COB suggested the milk was still of satisfactory quality.

Table 7: Evaluation of Measurements of Conductivity (Co) against pH, Titratable Acidity (TA), Alcohol Stability Test (AST), Clot on Boiling Test (COB), Rezazurin and Methylene Blue Reduction tests (MB) in Raw Milk Incubated at 30°C.

Time (h)	Co (mS/Cm)	pH	TA (%)	AST (70%)	COB	Rez (10min)	MB (R/Time)
0	4.12	6.76	0.160	(-)	(-)	6	>8.00
12	3.92	6.69	0.183	(-)	(-)	4	2.25
14	4.31	6.55	0.192	(+)	(-)	3	1.38
16	4.48	6.41	0.230	(+)	(+)	2	0.58
18	4.90	6.31	0.237	(+)	(+)	1	0.27

Trials in LP-s activated UHT Milk Inoculated Singly With Pure Strains

The general relationship between Co and bacterial counts (Table 8) demonstrates a slight initial decrease in Co followed by its increase as the counts increased. It has been established in earlier experiments that once spoilage has set in, the Co is greater than the initial Co value and increases consistently to a maximum Co value. The steady increases in Co to values greater than the initial Co value were observed at 25 h KQ for the control and for the LP/GE/E samples and at 35 h KQ for the LP/SCN^-/H_2O_2 sample corresponding to 7.4, 6.7, and 7.3 log cycles respectively. The LP/I^-/H_2O_2 treated sample did not show any measurable increase in Co above the initial Co value which reflects the acceptable quality of the sample up to the end of the experiment

Table 8: Change in conductivity (Co) over time (h) of Staphylococcus aureus inoculated UHT milk and Preserved Using Different LP-s at 37°C. (nd = not determined)

Time (h)	Control		LP/GE/E		LP/SCN^-/H_2O_2		LP/I^-/H_2O_2	
	Co (mS/Cm)	log cfu/ml	Co (mS/Cm)	log cfu/ml	Co (mS/Cm)	log cfu/ml	Co (mS/Cm)	log cfu/ml
0	4.67 ± 0.02	2.4	4.55±0.02	2.30	4.76 ±0.0	2.2	4.76 ± 0.03	1
5	4.63 ± 0.01	4.1	4.57 ± 0.01	3.70	4.78 ± 0.0	2.2	4.79 ± 0.0	<1
10	4.69 ± 0.01	5.8	4.61 ± 0.0	4.98	4.78 ± 0.0	3.7	4.81 ± 0.01	<1
15	4.65± 0.02	6.4	4.50 ± 0.0	5.30	4.68 ± 0.01	4.3	4.68 ±0.01	<1
25	4.70 ± 0.01	7.4	4.67 ± 0.01	6.70	4.70 ± 0.03	6.3	4.67 ± 0.03	<1
30	4.77 ± 0.04	8.4	4.75 ± 0.01	8.00	4.77 ± 0.03	6.8	4.66 ± 0.05	<1
35	4.78 ± 0.0	nd	4.80 ± 0.04	8.30	4.85 ±0.01	7.3	4.72 ±0.0	<1
40	4.89 ±0.07	nd	4.87 ± 0.05	8.40	4.92 ± 0.01	7.9	4.74 ± 0.01	<1
45	5.11 ± 0.01	nd	4.92 ± 0.03	nd	4.96 ± 0.01	nd	4.73 ± 0.02	<1

The decrease in E_h to a minimum value was consistent with increases in bacterial counts (Table 9). The negative E_h for the Control, LP/GE/E and LP/SCN^-/H_2O_2 preserved samples were recorded at 7.8, 7.6 and 7.3 log cycles respectively which corresponded with the KQ of 25 h, 25 h and 45 h. There was no negative drop in E_h in LP/I^-/H_2O_2 treated sample, the E_h was positive up to the end of the experiment implying that the milk was of an acceptable quality.

Table 9: Change in Redox potential (E_h) over time (h) of Pseudomonas aeruginosa inoculated UHT milk and preserved using different LP-s at 25°C. (nd = not determined)

Time (h)	Control		LP/GE/E		LP/SCN^-/H_2O_2		LP/I^-/H_2O_2	
	E_h (mV)	log cfu/ml	E_h (mV)	log cfu/ml	E_h (mV)	log cfu/ml	E_h (mV)	log cfu/ml
0	191± 8.49	2.6	171± 10.54	2.5	250 ±3.64	2.0	270 ± 2.97	<1
5	161 ± 5.8	3.4	116 ± 4.03	3.0	260 ± 1.27	1.4	260±18.67	<1
10	164± 1.77	5.5	136 ± 4.31	4.5	169 ± 0.07	<1	247 ± 1.27	<1
15	153± 3.54	6.4	139 ± 1.7	5.5	172 ± 2.4	<1	255±12.02	<1
25	-145±2.23	7.8	-58 ± 14.14	7.6	181±10.32	2.5	217 ± 12.4	<1
30	-344±13.4	8.2	-113±13.36	8.0	101 ± 0.42	3.5	175 ± 1.98	<1
35	-413±26.3	8.3	-312 ± 3.04	8.2	140 ± 4.17	5.5	152 ± 3.39	<1
40	-443±4.79	8.4	-387 ± 1.27	8.3	81 ± 0.49	6.5	174 ± 5.09	<1
45	-464±19.3	n.d	n.d	n.d	-138 ± 0.8	7.3	174 ± 7.07	<1
55	n.d	n.d	n.d	n.d	-212± 39.95	8.5	168 ± 4.17	<1

The DO varied as a function of bacterial counts, decreasing with increasing bacterial counts (Table 10). This was observed to drop below its detection level after 15 h for the control sample, 25 h for the LP/GE/E sample and after 30 h for the LP/SCN^-/H_2O_2 sample.

Table 10: Change in Dissolved Oxygen (DO) over time (h) of Bacillus cereus inoculated UHT milk and Preserved Using Different LP-s at 30°C. (n/d = not detected)

Time (h)	Control		LP/GE/E		LP/SCN^-/H_2O_2		LP/I^-/H_2O_2	
	DO%	log cfu/ml	DO%	log cfu/ml	DO%	log cfu/ml	DO%	log cfu/ml
0	53 ± 0.07	2.5	61 ± 4.74	2.4	58 ± 0.14	2.3	55 ± 0.71	2.26
5	39 ± 1.27	4.8	42 ± 1.34	3.6	51 ± 2.69	2.2	48 ± 1.2	1.6
10	33 ± 0.21	6.1	38 ± 2.26	5.0	39 ± 0.92	2.2	46 ± 3.11	<1
15	n/d	7.6	16 ± 0.57	6.5	39 ± 0.35	4.1	41 ± 1.7	<1
25	n/d	8.4	n/d	7.7	15 ± 0.78	5.4	18 ± 1.84	<1
30			n/d	8.2	n/d	6.9	23 ± 3.04	<1
35					n/d	7.4	25 ± 1.20	<1

Trials in LP-s Activated Raw Milk (On Field Application)

KQ estimates of LP activated raw milk using E_h and the control methods are presented and compared in Table 11. The KQ recorded by E_h show good agreement with the KQ estimates of both TA and AST in non-activated raw milk but E_h had the lowest KQ estimate for LP-s activated raw milk compared to all other methods. The lowest KQ estimate suggests early condemnation by E_h measurement which confirms the high sensitivity of E_h in quality prediction.

Table 11: Evaluation of the KQ estimates of E_h and the Control Methods in Untreated Raw Milk and LP Activated Raw Milk with SCN^-:H_2O_2 (20:10 mg/l) (results are expressed in hours taken for the end of shelf life to be reached)

Methods	Raw milk KQ (h)	LP-s activated raw milk KQ (h)
Decreases pH = 0.4	14	20
Increased acidity > 0.02	10	16
Unstable to 70% ethanol	10	18
Unstable to boiling	12	22
Negative E_h	10	14

The KQ estimates in non-activated LP-s raw milk were similar for all measurements (Table 12). It is likely that the spoilage was at a much more advanced stage which could be detected by all methods. However, the KQ for LP-s activated raw milk shows a much earlier detection of spoilage by TA compared to other methods. This was followed by Co and AST which showed similar sensitivity. The least sensitive methods were COB and pH.

Table 12: Evaluation of the KQ estimates of Co and the Control Methods in Untreated Raw Milk and LP Activated Raw Milk with SCN^-: H_2O_2 (15:15 mg/l) (results are expressed in hours taken for the end of shelf life to be reached)

Methods	Raw milk KQ (h)	LP-s activated raw milk KQ (h)
Decreased pH = 0.4	10	24
Increased acidity > 0.02	10	18
Unstable to 70% ethanol	10	20
Unstable to boiling	10	24
Conductivity > Initial value	10	20

Results for DO (Table 13) similarly show no differences in KQ estimates in the control sample (non activated LP-s raw milk) possibly due to the advanced stage of spoilage. However, in the LP-s activated raw milk, the AST and TA became positive before DO and proved consistently more sensitive in KQ estimates than DO. The KQ estimates for DO compared well with those for COB and pH.

Table 13: Evaluation of the KQ estimates of DO and the Control Methods in Untreated Raw Milk and LP Activated Raw Milk with SCN^-: H_2O_2 (15:15 mg/l) (results are expressed in hours taken for the end of shelf life to be reached)

Methods	Raw milkKQ (h)	LP-s activated raw milkKQ (h)
Decreased pH < 0.4	10	22
Increased acidity > 0.02	10	18
Unstable to 70% ethanol	10	20
Unstable to boiling	10	24
DO below detection limit	10	24

DISCUSSIONS

Initial values of DO, Co and E_h in Bulk and Individual Cow's Milk

The natural levels for DO, Co and E_h offer a baseline for assessing any changes due to either old age or contamination and hence the quality of milk. In this experiment the natural levels of these parameters in both individual cow's milk and bulk milk were determined. Results for E_h demonstrates close similarity in the mean E_h of bulk milk and individual cow's milk which reflects the dependence of bulk milk E_h on the E_h of individual samples. Bulk milk was obtained by mixing equal volumes of individual cow's milk. The mean E_h values were generally lower than literature data, that is, 200 to 300 mV (Sherbon, 1988) although the latter author demonstrated a wide range in E_h due to feeding effects.

The wide variations recorded for DO could be due to compositional variations. Shekar and Bhat (1984) observed significantly higher DO content in whole milk compared to that of skimmed milk of both buffalo and cow milk and significant variations between whole buffalo milk and whole cow milk but insignificant variations between their corresponding skimmed milks; they related these differences to variations in fat content. This was reflected by significantly higher DO content of buffalo milk fat than cow milk fat. Milking procedures (hand or machine milking), time and speed of stirring also affect the oxygen content of milk samples (Lück *et al.*, 1970). However, in this case all milks were machine milked. The mean DO reported in the literature (Shekar and Bhat, 1984) is 5.25 ppm. In their experiment, the milk was nevertheless covered on top with liquid paraffin to prevent further exposure to oxygen, which was not the case in the present study.

The Co determined in the present study compare well with available literature data, 4 to 5 mS Cm^{-1} (Sherbon, 1988), and 4 to 5.5 mS Cm^{-1}(Fox and McSweeney, 1998). Minerals, particularly Na^+, K^+ and Cl^- ,are the main contributors of electrical conductivity of milk (Sherbon, 1988) and greatly responsible for the variations. Fat contents can also reduce conductivity (by about 10%) through fat globules occupying volume and impending to the mobility of ion (Sherbon, 1988).

Preliminary trials on KQ

Under refrigeration condition, the rise in E_h was initially observed before decreasing over time. This initial rise in E_h was probably due to retarded microbial activity under refrigeration condition associated with continued diffusion of O_2 from the headspaces to the milk during storage (Schröder, 1982). A 5 ml headspace was left in the sample containers. The adoption and dominance of microbes after a prolonged storage period resulted in increased metabolic activities and the decline in milk E_h. It has been demonstrated (Oblinger and Kraft, 1973) that once bacteria have adapted to somewhat adverse environments, they posses the capacity to proliferate actively and attain large populations. It is also established that the lowest E_h is reached during the log phase of growth when the metabolic activities of the bacteria are most intense (Oblinger and Kraft, 1973). This depends on the reducing capacities of microbes and marks the end of shelf life. The rapid drop in E_h to negative values reflects the unpleasant state of milk. The E_h method demonstrated fairly rapid spoilage prediction, the time varying depending on the quality status of milk; from $\leq$10 minutes for normal unspoiled milk up to 30 minutes for milk with unsatisfactory quality, where E_h is negative. However, for routine testing the method is potentially

ideal, since it is not necessary to wait for a stable reading, as any negative value is indicative of poor quality.

Although the changes in DO content were inconsistent under refrigeration conditions, samples at 30°C showed a consistent decrease in DO to around zero. This coincided with spoilage as confirmed with the objective methods implying such a decrease in DO reflects spoilage. The cause of lack of consistency in the changes in DO observed during storage at 5°C is probably due to the metabolic flexibility of microbes and their variable O_2 uptake as they multiply (Schröder, 1982). Air in the container headspace also constitutes a potential source of O_2 available to the milk during storage (Schröder, 1982).

The increase in Co at spoilage is related to the increase in acid production and dissociation of the electrolytes (Sherbon, 1988) due to the metabolic conversion of uncharged or weakly charged organic molecules to charged products (Martin *et al*., 2003). The initial fall in Co on the other hand could be due to the buffering effects of milk and the neutralisation of the H^+ with OH^-, as the H^+ developed considerably further beyond the equivalent point of neutralisation the Co rose again. The sharp and consistent increase in Co observed in a starter culture enriched milk suggests that once spoilage has occurred, Co increases beyond the initial value and keeps on increasing until the maximum value is reached as determined by the degree of dissociation. Knowledge of the initial Co value could thus be importantly used as a basis for rejection or acceptance of milk. In terms of operation, the method is simple and the quickest of all three; not more than 2 min is required for a single measurement.
It is also important to point out the realisation that the very low Co value recorded on day 14 (i.e. 2.8 mS/Cm) for samples at 5°C was partly due to the depolarization of the Co sensor following its detachment from the meter for a prolonged time in the course of replacing one probe for another. A single meter was used for all three probes, as mentioned earlier and all parameters (DO, Co, E_h) were simultaneously analysed during preliminary trials. These interruptions were avoided in the subsequent experiments by using only one probe at a time.

Trials in Raw Milk
The decreases in DO to values close to zero or below detection limit were regularly observed and coincided with spoilage based on the objective methods. The DO at this level is thus regarded as indicative of the unsatisfactory quality of milk. This is consistent with previous findings (Schröder, 1982; Fox and McSweeney, 1998) which reported an almost complete consumption of DO towards the end of storage to a practically O_2 free milk. The consumption of DO gives an important indication of the keeping quality of milk. Rowe and Gilmour (1986) observed a drop in DO from 89% to < 10% towards the end of the log phase of growth of *Pseudomonas* spp, mesophiles and psychrotrophs. The increase in bacterial numbers increases the oxygen demand resulting in a large drop in DO. The earlier spoilage established by TA, AST, 10 min rezazurin and methylene blue tests than DO indicates a relatively similar leniency of DO as COB test.
As observed earlier, the drop in E_h to negative value indicates spoilage. Its quality estimates are consistent with that of TA, alcohol precipitation of milk and rezazurin

test. The late indication of poor quality by COB, pH and methylene blue tests confirms the quick response of E_h to changes in quality, from good to bad. The results agree fairly well with the previous experiment and demonstrate the potential of the method in KQ prediction.

Conductivity in a liquid medium including milk depends on the available net charges among the charged groups. The higher the net charge the greater the conductivity and vice versa. The milk Co higher than its initial Co value reflects the increase in microbial load to levels leading to unsatisfactory milk quality. Microbes are responsible for the breakdown of uncharged groups to charged groups. The coincident detection of spoilage by Co and AST, TA, rezazurin and methylene blue tests; shows the potential of the method for quality prediction. Similarly, earlier detection of spoilage than pH and COB signifies the higher sensitivity of the method in quality estimation

Trials in LP-s activated UHT Milk Inoculated Singly With Pure Strains

The variations in KQ recorded for samples preserved using different LP systems demonstrate how conductivity represents accurately the various degrees of dissociation / metabolisation. The increase in Co relies on the metabolic conversion of uncharged or weakly charged organic molecules (e.g. protein, lipids, and carbohydrates) to charged products such as amino acids, lactate and acetate and reflects metabolic activity (Martin *et al.*, 2003). The counts corresponding with the end of shelf life for the different samples show close similarity and suggest that noticeable increases in Co occurs at around 7 log cycles. The changes in Co at spoilage showed measurable increases of 0.03, 0.12 and 0.09 mS/Cm. These inconsistencies in the measurable increases in Co at spoilage may be accounted for by the metabolic activity and stage of spoilage. Lück *et al.* (1970) related such inconsistencies to unequal multiplication of the bacteria in different samples during incubation. The LP/I^-/H_2O_2 treated sample did not show any measurable increase in Co above the initial Co value which reflects the acceptable quality of the sample up to the end of the experiment.

The E_h method recorded spoilage when the bacterial counts were at around 7 log cycles. Lück *et al.* (1973) observed no detectable changes in E_h when the concentration of organism was between 5 to 6 log cycles. While Walstra and Jenness (1984) reported that significant changes in composition of milk are not detected until the bacterial counts has reached $\geq$ 6 log cycles. The value established here may however not reflect the real bacterial concentration at which changes in E_h may be detected in raw milk (due to different composition of bacterial flora) as each organism has a definite E_h value for growth or survival (Oblinger and Kraft, 1973). In their experiment they demonstrated that *Pseudomonas fluorescence* strains were unable to grow at E_h below +80 mV and Salmonella strains were unable to initiate growth at E_h levels below +30 mV. The blended E_h of mixed bacteria present in raw milk may be a useful indication of the bacterial population at which this could occur.

As was the case with Co, there was no negative drop in E_h in LP/I^-/H_2O_2 treated sample. The E_h was positive up to the end of the experiment implying that the milk

was of an acceptable quality. The log counts were similarly below detection limit which suggests higher antimicrobial effects of the LP/I^-/H_2O_2 system. Although, the microbial load that coincided with the negative E_h may seem to suggest that the E_h method is less sensitive to changes in E_h, these changes rely as well on the nature of micro-organism and substances produced that influence the potential (Eilers *et al.*, 1947).

The variation in time at which DO was below detection level shows the ability of DO to differentiate milks of different quality. The bacterial population which corresponded with undetectable DO levels varied between 6.9 and 7.7 log cycles. Rowe and Gilmour (1982) observed the drop in oxygen tension during the log phase of growth when the viable counts had reached 6 to 7 log cycles. The pattern of DO drop is consistent with previous findings (Schröder, 1982; Fox and McSweeney, 1998) which demonstrated an almost complete consumption of DO to a practically oxygen-free milk. Pickering and Jayne-Williams (1963) also reported a close relationship between viable counts and oxygen depletion. The rate of DO reduction varied with the amount of bacterial count which is an indication of the possible differentiation of bacterial quality. The consistent depletion of DO to below detection levels suggest that DO around zero reflects spoilage of milk. However, the study did not establish a precise minimum DO depletion level at which milk can be considered to be spoilt.

Trials in LP-s activated raw milk (Field trials)

The variation in KQ estimates established for the test methods and the objective methods proves the variation in sensitivity among different quality prediction methods (Tables 11-13). The good agreement in KQ estimates between E_h and both TA and AST in the control sample and the lowest KQ estimate for LP-s activated raw milk recorded for the E_h method (Table 11) show good reliability of E_h as a quality test method, especially for early indication of spoilage.

Co is almost as sensitive as the E_h, TA and AST and similarly more sensitive than both COB and pH as demonstrated by a much earlier detection of spoilage (Table 12).

DO is however, less sensitive than the AST and TA but compares well will COB and pH in terms of KQ estimates. The accurate comparison of its sensitivity is however complicated by lack of knowledge about the specific DO value at which spoilage can be established.

The variation in KQ recorded for the control samples in the different experiments during field trials could be due to the inconsistent in the quality of milk or to the fluctuations in storage temperatures and hence differences of the temperatures at which the samples were kept. The milk samples were stored at ambient temperature, 28-30°C during field trials. The sensitivities of the methods used have been shown to differ and this is clearly demonstrated in the LP-s activated raw milk.

On the other hand, the ability of the test methods to establish KQ for the differently preserved samples, confirms their potential in estimating the KQ of LP-s activated

milk and hence quality assurance in all variety of environment with little usage of chemical while acquiring results within the shortest possible time period.

In conclusion, it has been shown that the milk E_h, Co and DO offer suitable supplementary methods for milk quality prediction. All parameters are sensitive enough to differentiate the qualities of LP-s activated milk as for normal milk. E_h is the most sensitive and reliable parameter for quality prediction among the three parameters. The interpretation of its results is simple and provides accurate differentiation of milk quality. It's suitable for routine analysis as any negative E_h value indicates poor quality milk. The entire results show good reliability of E_h as a quality test method, especially for early indication of spoilage compared with most objective methods.

DO on the other hand, offers a good indication of the spoilage of milk although the minimum DO level at which milk should be considered spoilt is unclear. However, DO around zero or below detection limit can be used as a criteria for rejection of milk. The method is however less sensitive in KQ estimates compared to AST and TA but compares well with COB and pH.

Unlike other methods, the use of Co for quality prediction requires a prior knowledge of the initial Co value of milk under test which complicates its use in routine analysis. Milk is considered spoiled when the Co has consistently increased to a value greater than the initial Co value. For this reason, the method can only suit time course experiments.

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