Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Control 15 (2004) 217–229
FOOD CONTROL
www.elsevier.com/locate/foodcont

Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stored in different temperature conditions. A. Time–temperature indicators as quality-indicating tools

Maria Smolander [a,*], Hanna-Leena Alakomi [a], Tiina Ritvanen [b], Jukka Vainionpää [a], Raija Ahvenainen [a]

[a] *VTT Biotechnology, P.O. Box 1500, FIN-02044, VTT, Espoo, Finland*
[b] *Department of Chemistry, National Veterinary and Food Research Institute, EELA, P.O.Box 45, FIN-00581, Helsinki, Finland*

Received 9 January 2003; received in revised form 14 March 2003; accepted 17 March 2003

Abstract

The applicability of time–temperature indicators (TTIs) for the quality control of modified atmosphere packaged broiler chicken cuts was evaluated at various constant and variable temperature conditions. It was found that microbiological shelf-life could be considerably improved when the cold-chain was carefully maintained. Temperature had a critical effect on the amount of *Enterobacteriaceae*, proteolytic bacteria, hydrogen sulphide producing bacteria and clostridia, the microbial groups most likely to have an effect on the sensory quality. The results also indicate that TTIs seemed to be useful tools for evaluation of the quality of broiler chicken cuts.

Keywords: Time–temperature indicators; Freshness; Poultry

1. Introduction

The quality and shelf-life of raw, modified atmosphere (MA) packaged meat products depends on the storage temperature. Temperature control within the distribution chain plays a key role in maintaining the quality of these products on an adequate level throughout the selling period. As the temperature is decreased below the optimum for growth, generation times and lag times are extended. However, many of the major food spoilage and food poisoning microorganisms of concern are cold-adapted psychrotrophic bacteria which are able to grow at low temperatures, even approaching 0 °C (Russell, 2002). Cold-adapted bacteria grow at chill temperatures at rates that are equivalent to or not much slower than mesophiles at room temperatures. In addition, for psychrotrophic microorganisms it is typical that they can grow rapidly when temperatures rise to e.g. warm room temperature.

Time–temperature indicators (TTIs) can be used to help control the realisation of an unbroken cold-chain, since the indicator shelf-life is dependent on the time–temperature history of the package throughout the whole distribution chain. TTIs attached to the package surface integrate the cumulative time–temperature history of the product starting from the moment of indicator activation. The integrated time–temperature history is visualised as a colour change or colour movement. The change of the indicator proceeds as a function of time, the rate of visible colour change being proportional to the temperature. The complete change (end-point) of the indicator is reached when the indicator together with the package has been subjected to a certain, pre-defined heat-load (Fu & Labuza, 1995; Selman, 1995; Taoukis, Fu, & Labuza, 1991). Three different types of TTIs are already commercially available. Fresh-Check by LifeLines Technology, Inc. (USA) is based on polymerisation reaction of diacetylenic monomers. As the polymerisation takes place the colour of a ''bulls-eye'' patterned indicator changes gradually. TTI of VITSAB (Sweden) is based on an enzymatic reaction causing pH-change in the reaction mixture. VITSAB-TTI is activated by breaking the seal between a

* Corresponding author. Tel.: +358-9-456-5836; fax: +358-9-455-2103.
E-mail address: maria.smolander@vtt.fi (M. Smolander).

0956-7135/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0956-7135(03)00061-6

solution containing lipolytic enzyme and its lipid substrate. The reaction is visualised with a pH dye included in the system, the colour of the dye is changed from green to yellow as the pH changes during the reaction. 3M (USA) has recently launched a new TTI. It is manufactured in label form and it is activated upon application. In the activation two tapes are brought together and as the viscoelastic material from the first tape moves into the receptor area of the other tape (a time/temperature dependent process) the light transmission gradually increases revealing the colour underneath.

The application of commercially available TTIs in the quality control of various products has been studied and published by many research groups. For example, Chen and Zall (1987) successfully estimated the degree of freshness of dairy products with TTIs at constant temperatures. Fu, Taoukis, and Labuza (1991) studied the applicability of TTIs for spoilage monitoring of dairy products at variable temperatures with the aid of predictive microbiology. Riva (1997) studied the application of TTIs for shelf-life prediction of crescenza cheese. He found a good correlation between the kinetic characteristics of TTIs and the degradative reactions in cheese. Singh and Well (1987) applied enzyme-based TTIs to monitor quality changes of frozen strawberries. Boxtel and Sterrnburg (1997) studied the correlation between meat quality and TTIs.

The objective of this work was to study the applicability of TTIs for the sensory and microbiological quality control of MA packaged broiler chicken cuts at various constant and variable temperature conditions.

2. Methods

2.1. Packaging and storage of poultry samples

The study was carried out using broiler chicken cuts, which were obtained directly from the production plant and packaged at VTT Biotechnology one day after slaughtering. Three individual test runs A, B and C were performed. The broiler chicken cuts were obtained from two manufacturers, from one producer for test run A and from another for test runs B and C.

Broiler chicken cuts were packaged in 115 ± 5 g aliquots in 210 ml tray-packages (Dyno, HDPE). The packages were gas-flushed (80% CO_2 + 20% N_2) and sealed using a Dyno 462 VGA-machine (lid material Opalen HB65, UPM Pack, Valkeakoski, Finland).

For each time point of quality evaluation three replicate packages were packaged for microbiological examination and 10 replicate packages for sensory analysis. TTI labels were attached to the surface of the package lid of three replicate packages. For each quality evaluation point, frozen (storage at −20 °C) reference samples were prepared for use in the determination of sensory quality.

Broiler chicken cuts were stored in the dark at different constant and variable temperatures, the variable temperatures were simulating different favourable and unfavourable temperature conditions potentially taking place in the distribution chain (Fig. 1). Temperatures were measured with Tinyview Plus Gemini Data Loggers. The loggers were calibrated and correlated annually against a reference meter calibrated in accredited calibration laboratory. Uncertainty of the measurement was also calculated as part of the calibration and was found to be 0.05 °C for all loggers. A datalogger was

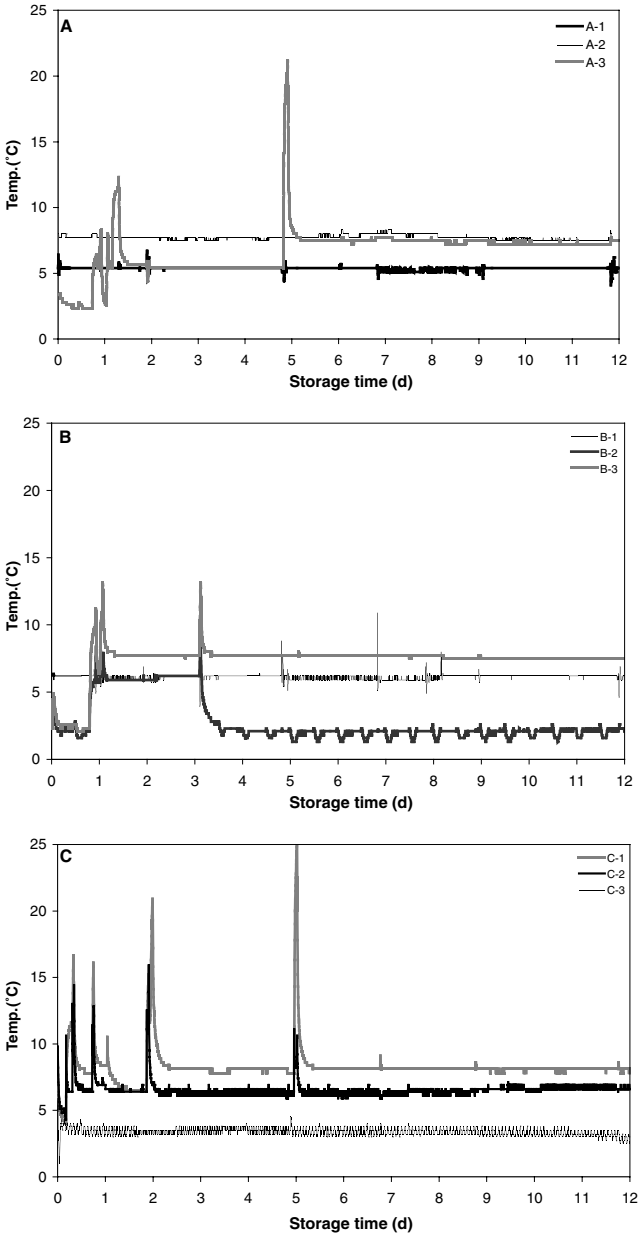

Fig. 1. Constant and variable temperature schemes in test runs A, B and C.

kept in close proximity of the samples and temperature was recorded in 5 min intervals throughout the storage. In variable temperature schemes the temperature varied from +3 °C representing the storage conditions at the processing plant up to 2 h exposure to +22 °C representing the extreme abuse during the transport from the retailer to consumer's refrigerator. The average temperatures measured during the first 12 d of the storage tests are shown in Table 1.

During the shelf-life study, the quality of the broiler chicken cuts was evaluated as a function of time. The time points for the evaluation were 0 d (= immediately after packaging) and after 5, 7, 9 and 12 d of storage.

2.2. Determination of microbiological quality

The microbiological quality of the broiler chicken cuts was determined from three replicate packages. Each broiler cut sample with original particle size of approximately 1–10 g was cut with sterile scissors into smaller particles with approximately 0.5–1 cm diameter and mixed thoroughly. Ten grams of this material was weighed and mixed with 90 ml sterile peptonesaline. The mixture was homogenised with Stomacher mixer (Seward, London, UK). Duplicate surface plates for each culture medium were prepared from appropriate serial dilutions unless otherwise stated. The media and incubation conditions were: for aerobic mesophilic bacteria Plate Count Agar (Difco, Detroit, MI, USA), 30 °C, 3 d; for aerobic psychrotrophic bacteria Plate Count Agar (Difco), 7 °C, 10 d; for lactic acid bacteria de Man, Rogosa, Sharpe Agar with pH adjusted with sorbic acid (pH 5.8) (Oxoid, Hampshire, UK), poured plates, 25 °C, 5 d, anaerobic jars with Anaerocult A®-strips (Merck, Darmstadt, Germany); for yeasts and molds, oxytetracycline glucose yeast extract agar with SR73 supplement, (Oxoid), 25 °C, 5 d; for *Enterobacteriaceae* Violet Red Bile Glucose Agar (LABM, Lancashire, UK), poured plates, 37 °C, 1 d; for coliforms Chromocult® Coliform Agar (Merck, Darmstadt, Germany), 37 °C, 2 d; for hydrogen sulphide producing bacteria Peptone Iron Agar supplemented with 0.05% cysteine, poured plates, 37 °C, 2 d; for anaerobic and facultatively anaerobic bacteria Tryptone Soya Agar supplemented with 5% horse blood (bioMeriéux, France), 37 °C, 2 d, anaerobic jars filled with mixed gas (85% N_2, 5% CO_2 and 10% H_2) by evacuation-replacement method (Anoxomat; Hart, Lichtenvoorde, The Netherlands); for *Brochothrix thermosphacta* streptomycin sulphate/thallium acetate/actidione agar (Oxoid), 25 °C, 2 d; for proteolytic micro-organisms citrate buffered calcium caseinate agar (Martley, Jayashankar, & Lawrence, 1970), 30 °C, 3 d; and for anaerobic sporeforming bacteria (sulphite reducing clostridia) Iron Sulphite Agar (Oxoid), poured plates, anaerobic jars filled with mixed gas (85% N_2, 5% CO_2 and 10% H_2) by evacuation-replacement method (Anoxomat; Hart, Lichtenvoorde, The Netherlands), 37 °C, 2 d. Confirmation of typical colonies was done according to standard methods.

2.3. Sensory evaluation

The sensory evaluation of the broiler chicken cuts was performed by evaluating the odour and the appearance using the profile method. Before the storage tests the profile was generated by the evaluators who listed quality descriptive properties of the chicken cuts. A selection of these descriptive properties were chosen for the profile. The intensity of the descriptive properties was evaluated using the scale from 0 to 10 (0 = very weak/no intensity, 10 = strong intensity). The descriptive properties used in the evaluations were total intensity of the odour, sweet odour, pungency, sulphuric odour, faultlessness (in test run A) or faultiness (in test runs B and C) of the odour, separation of the tissue fluid, eveness of the colour (test run A), intensity of the colour (test run A) and faultiness of the colour (test runs B and C).

Table 1
Storage temperatures for the broiler chicken cuts in test runs A, B and C

Test run/sample	Mean temperature, °C (0–12 d)	Remarks
A1	5.4	Constant temperature below statutory (in Finland)
A2	7.7	Constant temperature above statutory (in Finland)
A3	6.6	Variable temperature profile representing typical conditions in distribution chain from the producer to the consumer
B1	6.1	Constant temperature corresponding the highest statutory temperature (in Finland)
B2	2.9	Variable temperature profile representing ideal conditions in distribution chain from the producer to the consumer
B3	7.4	Variable temperature profile representing non-ideal conditions in real distribution chain from the producer to the consumer
C1	3.4	Constant temperature well below statutory, often recommended by the manufacturer
C2	6.5	Variable temperature profile representing typical conditions in real distribution chain from the producer to the consumer
C3	8.3	Variable temperature profile representing distribution chain with clearly broken cold chain

Eight to 10 judges who had been trained for the particular evaluation performed the sensory evaluation of the broiler chicken cut samples. The samples were tempered for 45 min at room temperature to 15 °C before the evaluation and they were presented to the judges in random order.

2.4. Time–temperature indicators

VITSAB (VITSAB), Fresh-Check (LifeLines) and 3M TTIs were studied in the experiment. In test run A 2–3 indicators of each type with slightly different reaction rates/times were attached to each package. In test runs B and C, 1–2 indicators of each type were used. TTIs were attached to the surface of the packages at +4 °C (corresponding temperature conditions in the production hall). The colour of the indicators was estimated visually and instrumentally at each quality analysis point.

The colour change of VITSAB TTIs was measured by Minolta Chroma Meter and the colour change of Fresh-Check and 3M TTIs was measured by X-Rite densitometer (model 404).

The colour change measured with Minolta Chroma Meter was expressed as the index of total colour change (ΔE) calculated with formula (1) using the colour immediately after packaging as the reference colour.

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad (1)$$

The colour change of the indicators was also visually estimated and evaluated using a scale from 1 to 3:

1 = indicator colour lighter than reference circle/indicator colour green,

2 = indicator colour same as the reference circle/indicator colour between green and yellow,

3 = indicator colour darker than the reference circle/indicator colour yellow.

3. Results and discussion

3.1. Microbiological quality

Aerobic mesophilic bacterial counts (AMBC) and the counts of lactic acid bacteria (MRS-S), *Enterobacteriaceae* (VRBGA), proteolytic bacteria, hydrogen sulphide producing bacteria and *Brochothrix thermosphacta* of

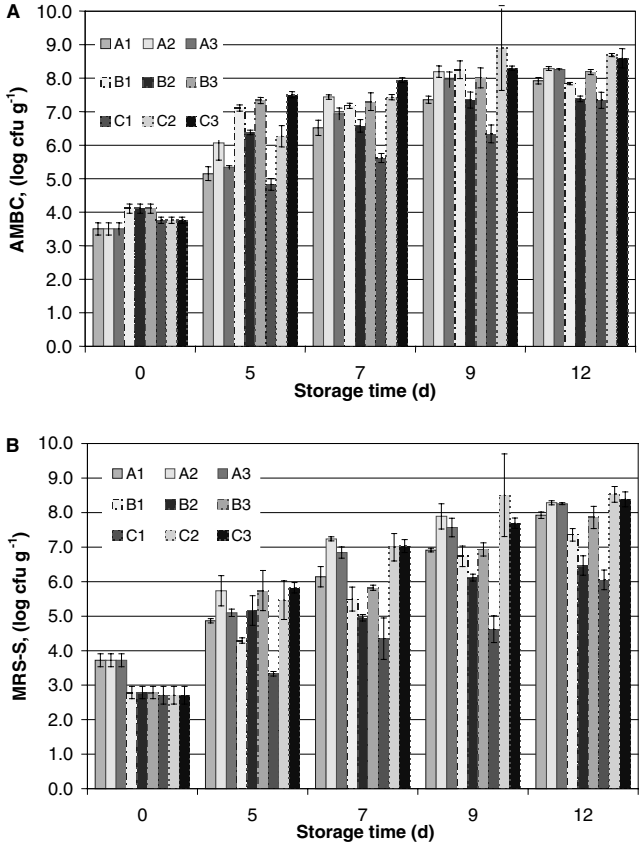

Fig. 2. The AMBC (A) and the number of lactic acid bacteria (MRS-S) (B) in MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1).

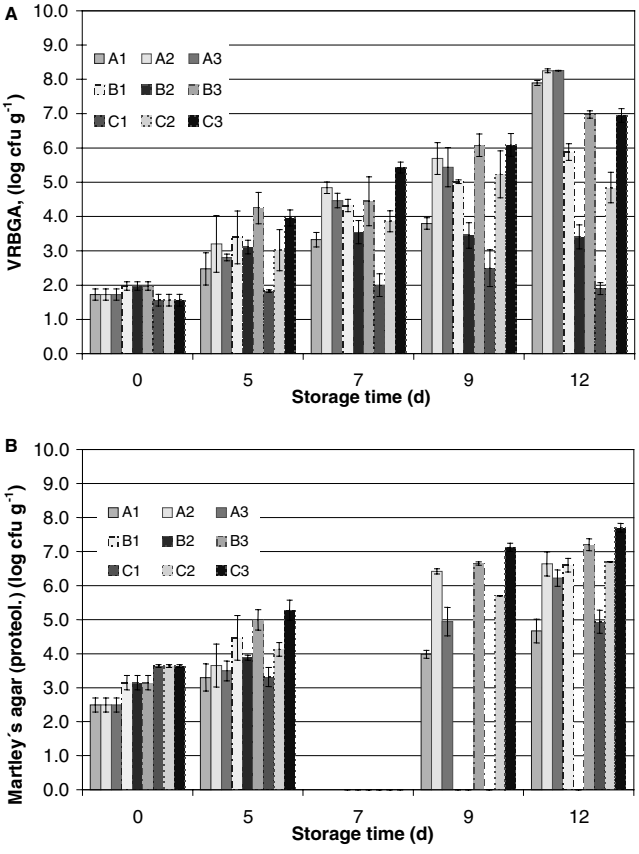

Fig. 3. The number of *Enterobacteriaceae* (VRBGA) (A) and proteolytic bacteria (Martley's agar) (B) in MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1).

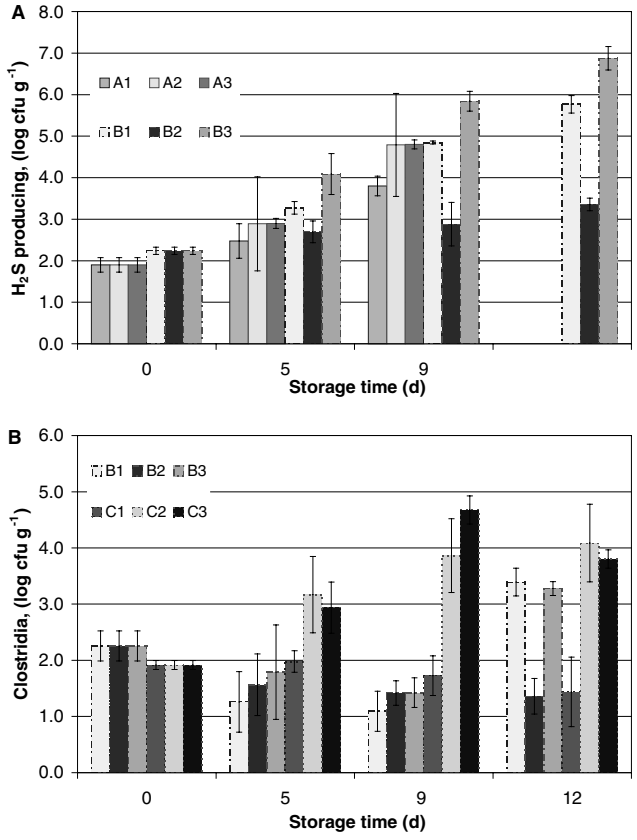

Fig. 4. The number of hydrogen sulphide producing bacteria (A) and clostridia (B) in MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1).

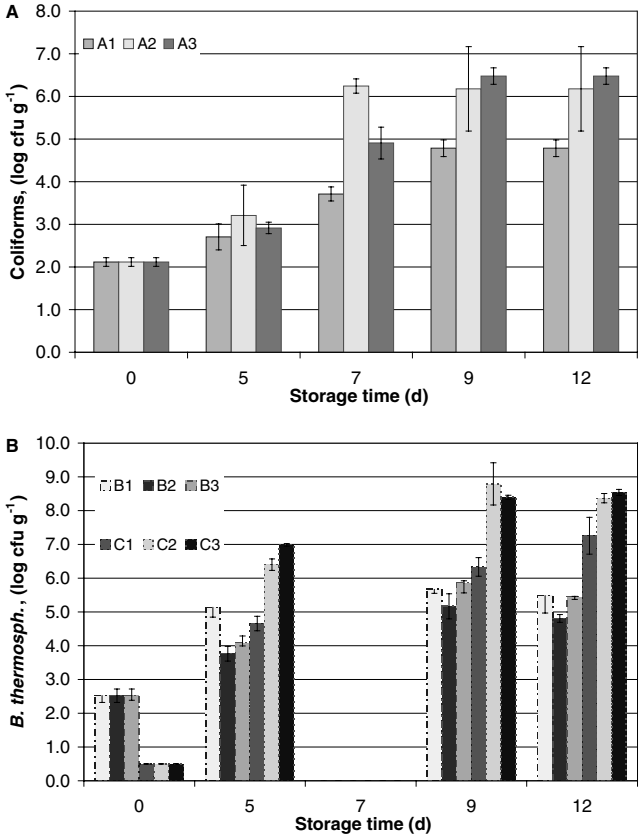

Fig. 5. The number of coliforms (A) and *Brochotrix thermosphacta* (B) in MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1). Detection limit log 1 cfu/g.

the broiler chicken cuts during the storage tests are shown as a function of time in Figs. 2–5, the results are averages of three replicate samples with standard deviations.

3.1.1. Aerobic bacteria and lactic acid bacteria

AMBC was approximately 10^4 cfu g^{-1} in the beginning of all the storage tests (Fig. 2(A)). Most bacteria contributing the aerobic plate count were psychrotrophic bacteria since the amount of the aerobic bacteria grown at +7 °C was nearly identical to AMBC (results not shown). The majority of the spoilage microorganisms belonged most likely to the groups of facultatively anaerobic bacteria (not shown) since this group behaved as a function of time and temperature in very similar way with AMBC. In the beginning of the storage test the numbers of lactic acid bacteria were slightly higher in test run A (log 3.7) than in the other two test runs B and C (log 2.8). Growth of lactic acid bacteria (MRS-S, Fig. 2(B)) was hindered in test runs B2 and C1 in which the storage temperature was constantly below 3.4 °C or the temperature profile followed the ideal conditions in distribution chain. In temperature profiles above 6 °C and especially in cases where the initial lactic acid bacteria count was high the MRS-S agar counts increased in 7 d from log 3.8 to log 7, indicating that also lactic acid bacteria were an important part of the spoilage flora.

At all temperatures studied in the three test runs aerobic mesophilic and psychrophilic counts increased steadily until microbial counts of log 8 were obtained (after 9 d storage in most cases). After that the level of AMBC remained stable. It was found that aerobic mesophilic and psychrotrophic counts were not remarkably affected by the storage temperature except in case of the constant low storage temperature C1 (mean + 3.4 °C) which restricted considerably the increase of total aerobic mesophilic count at least until 9 d. During the last three days of storage (9–12 d) the difference between the samples stored at C1 temperature and the higher temperatures was diminished. At favourable variable temperature (B2) microbiological growth was not retained as effectively as at constant low temperature (C1). This is most likely due to the higher original number of AMBC in B2 as well as due to the fact that in B2 the mean temperature during the first 5 d was 4.1 °C.

The quality of the broiler samples was evaluated according to criteria defined by Nordisk Ministerråd. According to this criteria the product has good quality if aerobic mesophilic plate count is below 10^6 cfu/g;

satisfactory quality if AMBC is between 10^6–10^7 cfu/g and unacceptable quality if AMBC is above 10^7 cfu/g. Also in earlier studies it has been reported that poultry products are considered unfit for consumption when AMBC reach 10^7 cfu/g (Senter, Arnold, & Chew, 2000). According to these limits the microbiological quality of the broiler cuts was unacceptable already after 5 d of storage at the constant +6.1 °C (B1) and at the unfavourable variable temperatures B3 and C3. At most storage temperatures the quality was found to be deteriorated after 9 d storage, except in case of constant low storage temperature C1 where the limit 10^7 cfu/g was exceeded only after storage for 12 d. This means in practice a difference of 7 d in the shelf-life when the difference in the mean temperature is 5 °C. Since the two samples with shortest and longest shelf-life were originating from same test run the difference is due to the storage temperature only, not due to the differences in the original microbial flora.

3.1.2. Enterobacteriaceae

At the beginning of storage the count of *Enterobacteriaceae* was approximately 10^2 cfu g^{-1} in all test runs. The effect of storage temperature was more pronounced on the number of *Enterobacteriaceae* than on the aerobic mesophilic and psychrotrophic counts (VRBGA, Fig. 3(A)). At constant, low temperature with mean + 3.4 °C (C1) the *Enterobacteriaceae* increased only 1 log during the 12 d storage period. Also at variable temperature with average + 2.9 °C (B2) the growth was below 2 log. The growth of *Enterobacteriaceae* was also retarded during the first 9 d of storage at constant temperature with average + 5.4 °C (A1). At temperatures +6.1 °C or higher no delay in the growth of *Enterobacteriaceae* was observed. The count of *Enterobacteriaceae* was 10^4 cfu/g already after 5 d of storage at the highest temperatures (B3 and C3). The final counts after 12 d of storage were as high as 10^6–10^8 cfu/g in most of the studied temperatures above +5.4 °C. *Enterobacteriaceae* are facultatively anaerobic Gram negative bacteria which are known to be able to rapidly adapt to the changing environments.

3.1.3. Proteolytic bacteria

Another group of bacteria clearly retarded by low temperature was proteolytic bacteria growing on Martley's medium and forming a precipitation of casein. Total number of microbes growing on Martley's media was highly consistent with AMBC (data not shown).

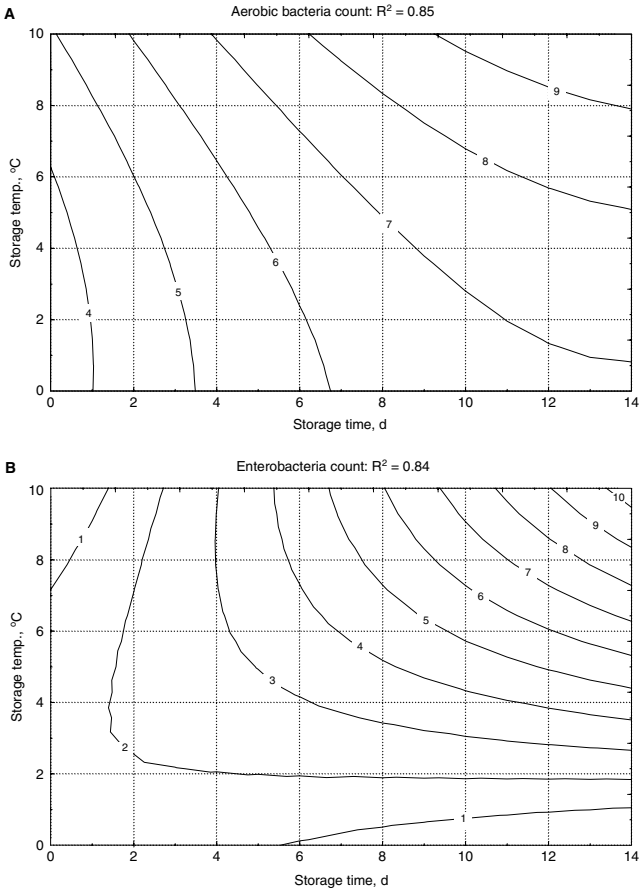

Fig. 6. The effect of time and temperature on the aerobic plate count (A) and *Enterobacteriaceae* (B).

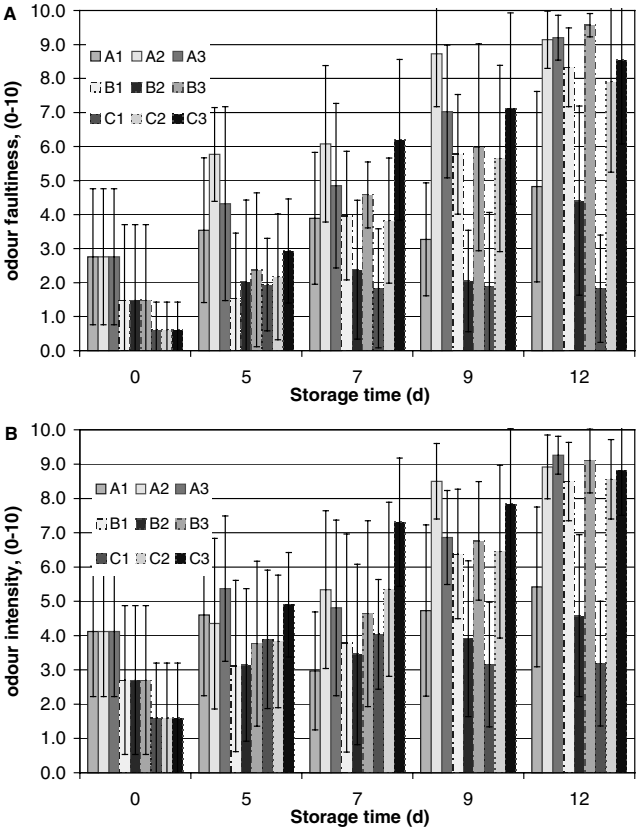

Fig. 7. Odour faultiness (A) and intensity (B) of MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1).

The determination of proteolytic microbes was not performed at all temperatures throughout the work; nevertheless, it was evident that their growth was dependent on the temperature—a clear retardation took place at temperatures A1 (+5.4 °C) and C1 (+3.4 °C) (Fig. 3(B)).

3.1.4. Hydrogen sulphide producing bacteria

Hydrogen sulphide producing bacteria (IA) were studied in test runs A and B. Their growth was also restricted by an unbroken cold chain (Fig. 4(A)). For instance after 12 d of storage in test run B the amount of hydrogen sulphide producing bacteria was more than two times higher at storage temperature B3 (+7.4 °C) than at storage temperature B2 (+2.9 °C). The difference was also reflected in the amount of hydrogen sulphide produced in the package head space (Rajamäki et al., submitted).

3.1.5. Clostridia

In test run A the clostridia remained below 10^1 cfu/g. In test runs B and C clostridia originally approximated 10^2 cfu/g (Fig. 4(B)). During the storage the growth of clostridia was very clearly retarded by low temperatures B2 and C1 (+2.9 and +3.4 °C).

3.1.6. Coliforms

Coliform counts were determined in test run A. They remained low ($<10^4$ cfu g^{-1}) during the first 7 d of storage when the mean temperature was +5.4 °C (Fig. 5(A)). After the same storage period at higher temperature (+7.7 °C) the number of coliforms was above 10^6 cfu/g.

3.1.7. Brochothrix thermosphacta

Brochothrix thermosphacta is a remarkable spoilage organism of vacuum and MA packaged products. It is known to produce compounds like acetoin, acetic acid, isobutyric, isovaleric and 2-methylbutyric acids The growth of *B. thermosphacta* was studied in test runs B and C. It was clearly restricted at +3.4 °C in test run C (Fig. 5(B)). In general growth was slower in test run B even though numbers of the bacteria were higher at the beginning of storage. In sample C the level of *B. thermosphacta* was below the detection limit (<log 1) at the beginning of storage. In test run B the growth of lactic

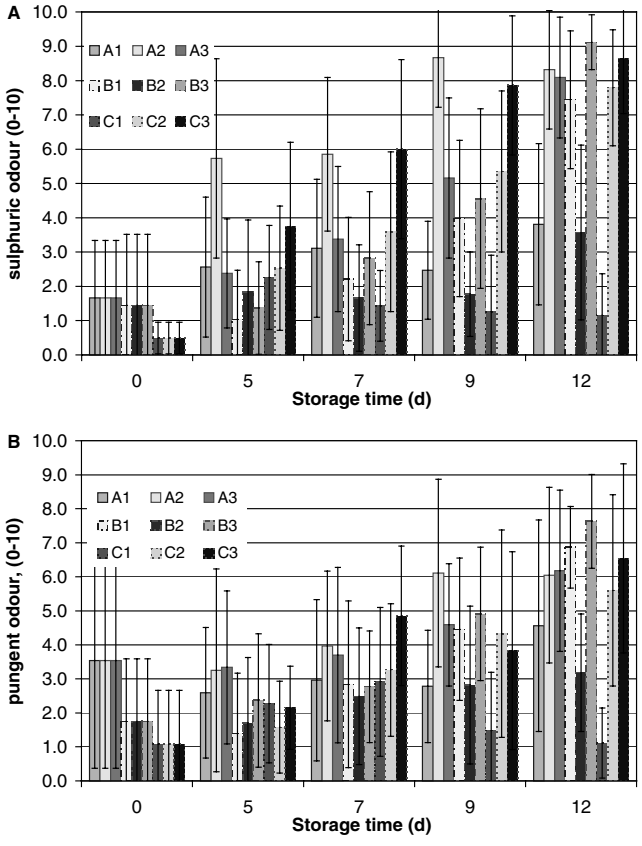

Fig. 8. Sulphuric (A) and pungent (B) odour of MA packaged broiler chicken cuts as a function of time at different storage temperatures (as described in Table 1).

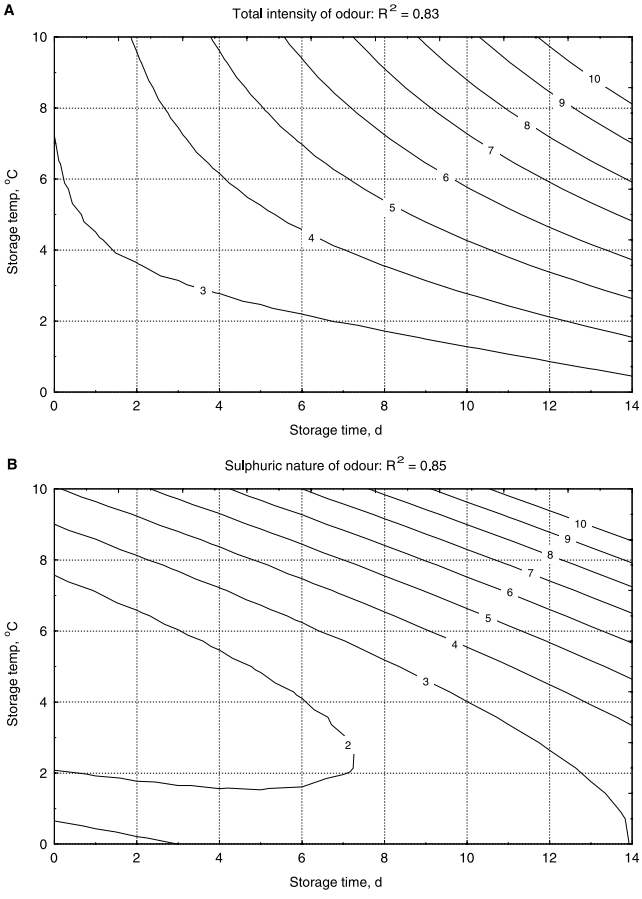

Fig. 9. The effect of time and temperature on the odour intensity (A) and sulphuric odour (B).

Table 2
Correlation between the microbiological and sensory quality of broiler chicken cut (test runs B and C)

	Total intensity		Pungency		Sulphuric odour		Faultiness		Sweet odour	
	B	C	B	C	B	C	B	C	B	C
Aerobic mesophilic bacteria	0.680 $p = 0.010$	0.843 $p = 0.000$	0.623 $p = 0.023$	0.721 $p = 0.004$	0.556 $p = 0.048$	0.808 $p = 0.000$	0.655 $p = 0.015$	0.828 $p = 0.000$	0.598 $p = 0.031$	0.733 $p = 0.003$
Aerobic psychrotrophic bacteria	0.619 $p = 0.024$	0.815 $p = 0.000$	0.567 $p = 0.043$	0.687 $p = 0.007$	0.508 $p = 0.076$	0.782 $p = 0.001$	0.624 $p = 0.023$	0.797 $p = 0.001$	0.570 $p = 0.042$	0.676 $p = 0.008$
Enterobacteriaceae	**0.923** **p = 0.000**	**0.901** **p = 0.000**	**0.899** **p = 0.000**	0.823 $p = 0.000$	**0.853** **p = 0.000**	**0.933** **p = 0.000**	**0.910** **p = 0.000**	**0.876** **p = 0.000**	0.749 $p = 0.003$	0.547 $p = 0.043$
Lactic acid bacteria	**0.858** **p = 0.000**	**0.876** **p = 0.000**	0.836 $p = 0.000$	0.782 $p = 0.001$	0.792 $p = 0.001$	**0.855** **p = 0.000**	0.842 $p = 0.000$	**0.864** **p = 0.000**	0.763 $p = 0.002$	0.720 $p = 0.004$
Anaerobic and facultatively anaerobic bacteria	0.729 $p = 0.005$	0.826 $p = 0.000$	0.689 $p = 0.009$	0.716 $p = 0.004$	0.630 $p = 0.021$	0.782 $p = 0.001$	0.741 $p = 0.004$	0.817 $p = 0.000$	0.676 $p = 0.011$	0.733 $p = 0.003$
B. thermosphacta	0.635 $p = 0.020$	−0.456 $p = 0.101$	0.590 $p = 0.034$	*−0.328* *p = 0.253*	0.512 $p = 0.074$	*−0.341* *p = 0.233*	0.624 $p = 0.023$	*−0.352* *p = 0.217*	0.650 $p = 0.016$	*−0.448* *p = 0.108*
Martley's agar (total)	0.696 $p = 0.008$	0.828 $p = 0.000$	0.644 $p = 0.018$	0.713 $p = 0.004$	0.580 $p = 0.038$	0.792 $p = 0.001$	0.699 $p = 0.008$	0.816 $p = 0.000$	0.640 $p = 0.018$	0.720 $p = 0.004$
Martley's agar (proteolytic)	**0.906** **p = 0.000**	**0.903** **p = 0.000**	**0.880** **p = 0.000**	0.807 $p = 0.000$	0.825 $p = 0.001$	**0.934** **p = 0.000**	**0.896** **p = 0.000**	**0.906** **p = 0.000**	0.780 $p = 0.002$	0.647 $p = 0.012$
H_2S-producing bacteria	**0.917** **p = 0.000**		**0. 879** **p = 0.000**		**0.856** **p = 0.000**		**0.923** **p = 0.000**		0.771 $p = 0.002$	
Yeast and moulds	0.804 $p = 0.001$	*−0.393* *p = 0.165*	0.830 $p = 0.000$	−0.520 $p = 0.057$	**0.895** **p = 0.000**	*−0.354* *p = 0.215*	0.817 $p = 0.001$	*−0.415* *p = 0.140*	0.570 $p = 0.042$	*−0.298* *p = 0.301*
Sulphite reducing clostridia	*0.321* *p = 0.285*	0.738 $p = 0.003$	0.697 $p = 0.008$	0.619 $p = 0.018$	0.747 $p = 0.003$	0.789 $p = 0.001$	0.628 $p = 0.022$	0.669 $p = 0.009$	0.648 $p = 0.017$	*0.326* *p = 0.255*

Correlations with low ($p > 0.05000$) statistical significance are printed using italics. Good correlations above 0.85 have been emphasized with bold type.

acid bacteria seems to have restricted the growth of *B. thermosphacta*.

3.1.8. Statistical analysis

The effect of storage time and temperature on the AMBC and *Enterobacteriaceae* was also compared by creating contour plots shown in Fig. 6. The stronger effect of temperature on the counts of *Enterobacteriaceae* than on the aerobic plate count is clearly seen in the graphs.

3.2. Sensory quality

Sensory quality of the broiler chicken cuts was evaluated from raw product. Despite some variation between replicate samples it was clearly learned that the changes related to the storage time and temperature took place in the odour of the product. Some deterioration related to the appearance (colour) of the broiler cuts could also be seen, but the changes were not as clear and unambiguous as those related to the odour of the product.

It was demonstrated that in all test runs the total intensity, pungency, faultiness and sulphuric nature of the odour were clearly dependent on the storage temperature and time. These quality attributes are shown as a function of time in Figs. 7 and 8.

In the beginning of storage the total intensity, pungency and faultiness of the odour were highest in test run A. This was assumed to be due to the odourous compounds emitted from packaging material and the differences between the test runs were diminished during the storage. A remarkable restriction in the increase of faultiness, total intensity, sulphuric nature and pungency of the odour could be seen in all temperatures with mean 5.4 °C or less. At the end of storage a clear difference was seen already between temperatures 5.4 °C (A1) and 6.1 °C (B1).

The effect of storage temperature and time on the odour of the broiler chicken cuts was also studied with contour plots (Fig. 9). It can clearly be seen that if the temperature is maintained on a low level especially the sulphuric odour remains on a very low level.

3.3. Sensory quality vs. microbiological quality

The effect of storage time and temperature on the attributes of sensory odour of broiler chicken cuts was clearly consistent with the effect of temperature on the microbiological quality of the cuts.

When the correlations between the sensory quality and the microbiological quality were calculated, it could be seen that there was a high correlation between the sensory odour of the broiler cuts and *Enterobacteriaceae*, hydrogen sulphide producing bacteria and proteolytic bacteria (Table 2). In some cases a clear correlation was also found out between the odour and the number of yeasts and moulds and lactic acid bacteria.

The results obtained in this study indicate that even a small increase in temperature has a considerable effect on the growth of some micro-organisms like *Enterobacteriaceae*, hydrogen sulphide producing bacteria and proteolytic micro-organisms in poultry meat. According to our present understanding about these groups, they are most likely to have an effect on the deterioration of the odour of the chicken cuts by producing e.g. sulphuric compounds. Therefore the restriction of their growth has a remarkable effect on the maintenance of the sensory quality at an acceptable level. This is in good agreement with our earlier statement that if low temperature can be maintained the sulphuric odour of the chicken cuts remains at a very low level (Fig. 9). However, one should not forget microbial interactions. Gram et al. (2002) recently reviewed role of microbial interactions in the food spoilage. Also Borch, Kant-Muermans, and Blixt (1996) reported lactic acid and *Enterobacteriaceae* association and enhanced volatiles production.

3.4. Time–temperature indicators

Several different TTIs were tested preliminarily in test run A for their capability to indicate the microbiological and sensory quality of the broiler chicken cuts. Since the colour change of the indicators was evaluated both instrumentally and visually the correlation between the two methods was calculated (Table 3). It was found out

Table 3
Correlation between instrumentally and visually evaluated colour change of different TTIs

	Correlation coefficient statistical significance
Fresh-Check HG363	0.855 $p = 0.000$
Fresh-Check G269	0.870 $p = 0.000$
Fresh-Check JG2211	0.865 $p = 0.000$
VITSAB C2-10	0.967 $p = 0.000$
VITSAB C2-13	0.973 $p = 0.000$
VITSAB M2-15	0.970 $p = 0.000$
VITSAB M2-21A	0.956 $p = 0.000$
VITSAB M2-21B	0.970 $p = 0.000$

that the colour evaluation methods were comparable and also that the instrumental methods could be utilised when the correlation between the colour change of the indicator and quality of the broiler chicken cuts had been determined.

A good correlation could be seen between the colour of most of the indicators evaluated in test run A and the quality of the broiler chicken cuts (Table 4). High coefficients were obtained especially between the aerobic mesophilic count or the number of lactic acid bacteria and Fresh-Check indicators, 3M indicators and VITSAB indicators of C-type. The amounts of *Enterobacteriaceae* (VRBGA) correlated well with Fresh-Check indicators, 3M indicators and VITSAB indicators of M2-21-type. A correspondence between the odour of the broiler chicken cuts and the colour change of VITSAB indicators of M2-21-type was also seen. These correlations indicate that the activation energies of the TTIs were similar to the activation energies of the deterioration processes taking place in the broiler chicken cuts.

Table 4
Correlation between the colour of the TTIs and microbiological or sensory quality of broiler chicken cuts (test run A)

	AMBC	VRBGA	MRS-S	Pungent odour	Sulphuric odour	Odour faultiness	Odour intensity
Fresh-Check HG363/X-rite	**0.916** **p = 0.000**	**0.926** **p = 0.000**	**0.937** **p = 0.000**	0.815 $p = 0.001$	0.828 $p = 0.000$	**−0.854** **p = 0.000**	0.822 $p = 0.001$
Fresh-Check HG363/visual	0.723 $p = 0.005$	**0.858** **p = 0.000**	0.765 $p = 0.002$	0.804 $p = 0.001$	0.676 $p = 0.011$	−0.739 $p = 0.004$	**0.852** **p = 0.000**
Fresh-Check G269/X-rite	**0.946** **p = 0.000**	**0.949** **p = 0.000**	**0.964** **p = 0.000**	0.810 $p = 0.001$	0.798 $p = 0.001$	−0.835 $p = 0.000$	0.817 $p = 0.001$
Fresh-Check G269/visual	0.768 $p = 0.002$	**0.862** **p = 0.000**	0.801 $p = 0.001$	0.830 $p = 0.000$	0.661 $p = 0.014$	−0.715 $p = 0.006$	0.817 $p = 0.001$
3M-type3/X-rite	**0.889** **p = 0.000**	**0.938** **p = 0.000**	**0.919** **p = 0.000**	0.799 $p = 0.001$	0.784 $p = 0.002$	−0.809 $p = 0.001$	0.812 $p = 0.001$
3M-type4/X-rite	**0.905** **p = 0.000**	**0.880** **p = 0.000**	**0.921** **p = 0.000**	0.723 $p = 0.005$	0.765 $p = 0.002$	−0.763 $p = 0.002$	0.712 $p = 0.006$
Fresh-Check JG2211/X-rite	**0.928** **p = 0.000**	**0.930** **p = 0.000**	**0.946** **p = 0.000**	0.799 $p = 0.001$	0.837 $p = 0.000$	**−0.866** **p = 0.000**	0.806 $p = 0.001$
Fresh-Check JG2211/visual	**0.923** **p = 0.000**	**0.857** **p = 0.000**	**0.939** **p = 0.000**	0.751 $p = 0.003$	0.783 $p = 0.002$	−0.775 $p = 0.002$	0.649 $p = 0.016$
VITSAB C2 10/Minolta	**0.958** **p = 0.000**	0.758 $p = 0.003$	**0.928** **p = 0.000**	*0.491* *p = 0.089*	0.600 $p = 0.030$	−0.618 $p = 0.024$	*0.490* *p = 0.089*
VITSAB C2 10/Visual	**0.934** **p = 0.000**	0.689 $p = 0.009$	**0.905** **p = 0.000**	*0.448* *p = 0.124*	*0.542* *p = 0.056*	*−0.546* *p = 0.053*	*0.399* *p = 0.177*
VITSAB C2 13/Minolta	**0.955** **p = 0.000**	0.812 $p = 0.001$	**0.952** **p = 0.000**	0.577 $p = 0.039$	0.624 $p = 0.023$	−0.646 $p = 0.017$	*0.545* $p = 0.054$
VITSAB C2 13/visual	**0.956** **p = 0.000**	0.822 $p = 0.001$	**0.961** **p = 0.000**	0.670 $p = 0.012$	0.675 $p = 0.011$	−0.695 $p = 0.008$	0.613 $p = 0.026$
VITSAB M2 15/Minolta	0.810 $p = 0.001$	0.568 $p = 0.043$	0.750 $p = 0.003$	*0.286* *p = 0.344*	*0.487* *p = 0.091*	*−0.500* *p = 0.082*	*0.354* *p = 0.235*
VITSAB M2 15/visual	0.682 $p = 0.010$	*0.413* *p = 0.161*	0.610 $p = 0.027$	*0.137* *p = 0.655*	*0.373* *p = 0.209*	*−0.385* *p = 0.195*	*0.253* *p = 0.405*
VITSAB M2 21A/Minolta	0.671 $p = 0.012$	**0.907** **p = 0.000**	0.736 $p = 0.004$	**0.850** **p = 0.000**	0.779 $p = 0.002$	−0.831 $p = 0.000$	0.838 $p = 0.000$
VITSAB M2 21A/visual	0.678 $p = 0.011$	**0.896** **p = 0.000**	0.737 $p = 0.004$	**0.908** **p = 0.000**	0.767 $p = 0.002$	−0.844 $p = 0.000$	**0.900** **p = 0.000**
VITSAB M2 21B/Minolta	0.663 $p = 0.014$	**0.881** **p = 0.000**	0.717 $p = 0.006$	0.831 $p = 0.000$	0.754 $p = 0.003$	−0.817 $p = 0.001$	**0.857** **p = 0.000**
VITSAB M2 21B/visual	0.678 $p = 0.011$	**0.896** **p = 0.000**	0.737 $p = 0.004$	**0.908** **p = 0.000**	0.767 $p = 0.002$	−0.844 $p = 0.000$	**0.900** **p = 0.000**

Correlations with low ($p > 0.05000$) statistical significance are printed using italics. Good correlations above 0.85 have been emphasized with bold type.

Even if the correlation of the rates of quality and colour changes is good this is not sufficient. Also the end point of the indicator has to correspond the end-point of the product quality. For this purpose the microbiological shelf-life of broiler chicken cuts was determined on the basis to the number of aerobic bacteria with 10^7 cfu/g as a limiting value for a microbiologically acceptable product. The sensory shelf-life was defined to continue as long as none of the attributes of sensory quality obtained a value of 6 or more (Table 5).

Using these criteria both sensory and microbiological shelf-lives were 9 and 7 d at the two higher temperatures +6.6 and +7.7 °C, respectively. At the lowest temperature the sensory quality was still acceptable after 12 d storage, but the amount of aerobic plate count had already slightly exceeded 10^7 cfu/g.

On the basis of the shelf-life correspondence and the correlation data two indicators, Fresh-Check JG221 and VITSAB C2-13 were used in further test runs B and C.

It was found out that even if there were some differences in the original quality of the broiler cuts a good correlation between aerobic plate count and the colour change of the indicators could still be obtained when the results from all three test runs were combined (Fig. 10). The end-point of both indicators corresponded to aerobic plate counts between 10^7 and 10^8 cfu/g. In addition to aerobic plate count, Fresh-Check indicator JG221 had a relatively good correlation with the counts of *Enterobacteriaceae* (VRBGA) and odour faultiness. The colour change of the indicator has been plotted against these quality attributes in Fig. 11. The end-point of the indicator corresponds to approximate values of 10^5 cfu/g and 5 for *Enterobacteriaceae* and odour faultiness, respectively. A relatively good correlation between product quality and indicator colour change can be seen even if the results originated from three individual test runs.

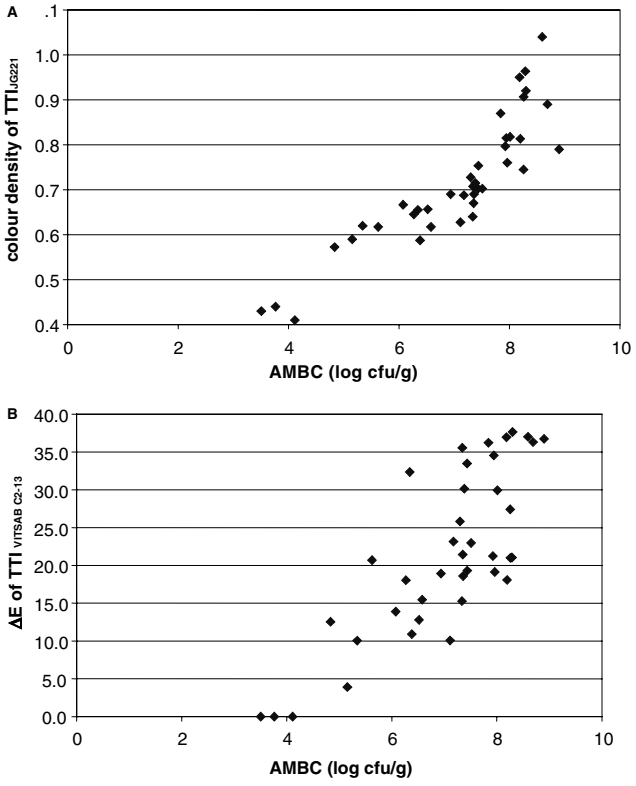

Fig. 10. Correlation between aerobic plate count of MA packaged broiler chicken cuts and the colour of Fresh-Check (A) or VITSAB (B) TTIs (results combined from three storage tests). Indicator end-points: colour density of 0.75 for Fresh-Check and ΔE of 20 for VITSAB.

4. Conclusions

On the basis of the microbiological results obtained in this study, it can be said that the microbiological shelf-life could be considerably improved when the temperature was maintained at a very low level representing ideal, unbroken cold chain. When the AMBC above

Table 5
Correspondence between visually evaluated end-point of TTIs and sensory/microbiological quality of broiler chicken cuts

Shelf-life of quality attr./indicator[a]	Storage temperature +5.4 °C	Storage temperature +6.6 °C	Storage temperature +7.7 °C
AMBC (log cfu/g >7)	9 d (log cfu/g = 7.35)	9 d	7 d
Sensory quality (One or more sensory attributes scored $\geqslant$ 6)	>12 d (several scores of 5 at 12 d)	9 d	7 d
Fresh-Check G269	12 d	12 d	9 d
Fresh-Check HG363	>12 d	>12 d	12 d
Fresh-Check JG221-1	**12 d**	**9 d**	**7 d**
VITSAB M2-15	5 d	5 d	5 d
VITSAB M2-21 a	>12 d	>12 d	12 d
VITSAB M2-21 b	>12 d	>12 d	12 d
VITSAB C2-10	7 d	7 d	7 d
VITSAB C2-13	**12 d**	**9 d**	**7 d**
3M (3)	>12 d	>12 d	>12 d
3M (4)	>12 d	>12 d	>12 d

[a] The first time-point with the evaluation as unacceptable.

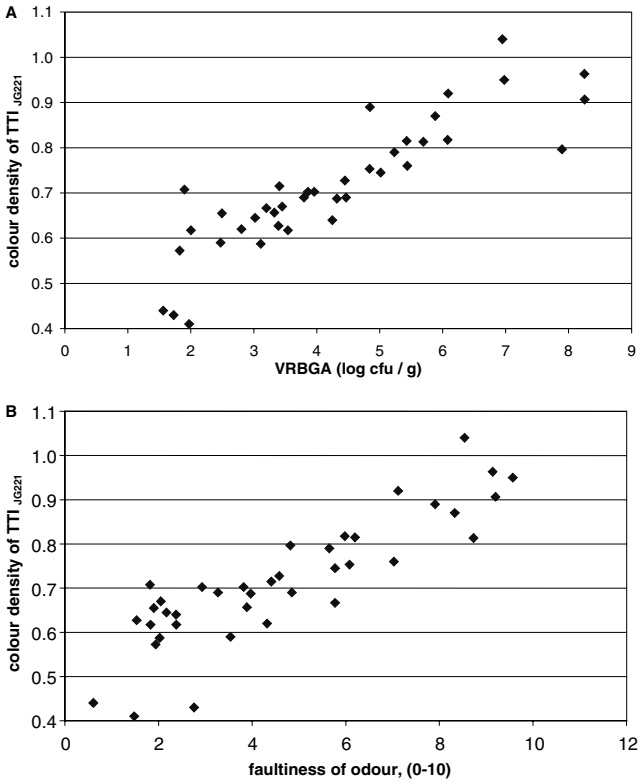

Fig. 11. Correlation between the amount of *Enterobacteriaceae* (A) or odour faultiness (B) of MA packaged broiler chicken cuts and the colour of Fresh-Check TTIs (results combined from three storage tests). Indicator end-point: colour density of 0.75.

10^7 cfu/g was used as a limit of acceptability of product, the shelf-life of the broiler chicken cuts was more than doubled when the average storage temperature was lowered from 8.3 to 3.4 °C. However even more critical effects were seen in the amount of *Enterobacteriaceae*, proteolytic bacteria, hydrogen sulphide producing bacteria and clostridia. The lowering of the temperature reduces even the growth rate of cold-adapted psychrotrophic bacteria. However, one should remember that shelf-life cannot be extended too far since this can give an opportunity for some pathogens like *Listeria* (able to grow near 0 °C) to multiply to too high level.

The strong effect of temperature on the sensory quality of broiler chicken cuts could also be seen despite the relatively high deviation between replicate samples. This result could be expected since the microbial groups whose growth is retarded by low temperature (e.g. *Enterobacteriaceae*) can produce sulphuric compounds having a pronounced role in the deterioration of sensory quality poultry meat (Dainty, 1996).

It should be pointed out that the AMBC often used as a quality criteria of meat products did not give a complete picture of the microbiological and sensory quality of MA packaged broiler chicken cuts during the storage at various temperature conditions. The effect of storage temperature was more clearly seen on the number of *Enterobacteriaceae*, which also corresponded with the sensory odour of broiler chicken cuts. It has earlier been stated that level of specific spoilage organisms (SSO) can be used to predict the remaining self life under which the SSO is important (Gram et al., 2002).

Since even a small increase in temperature has a critical effect on the quality of poultry meat the use of TTIs is highly justified. According to the results obtained the TTIs seemed to be useful tools for evaluation of the quality of broiler chicken cuts. The rate of colour change of most TTIs correlated especially well with the amount of aerobic plate count whereas some had a good correlation with the amount of *Enterobacteriaceae* and the odour of broiler chicken cuts. Some of the indicators correlated well with both aerobic mesophilic and psychrotrophic counts and *Enterobacteriaceae* counts. Even if the indicators were selected on the basis of their availability it was possible to find indicators with end-points matching the microbiological and sensory shelf-life of broiler chicken cuts. In real implementation of TTIs the shelf-life of the indicator can be tailored according the product shelf-life.

Acknowledgements

The project was initiated in 2000 as part of the On-line Measurement Techniques in Process Industry Technology Programme of the National Technology Agency (Tekes) in Finland. The work was financially supported by Tekes and Finnish food and packaging industry. The skillful assistance of the technical staff of VTT Biotechnology and EELA is gratefully acknowledged.

References

Borch, E., Kant-Muermans, -L., & Blixt, Y. (1996). Bacterial spoilage of meat and cured meat products. *International Journal of Food Microbiology, 33*, 103–120.

Boxtel, L. B. J. van, & Sterrenburg, P. S. (1997). Monitoring shelf life of meat and meat products by TTIs. In: Proceedings 43rd ICOMST, Auckland, New Zealand.

Chen, J. H., & Zall, R. R. (1987). Packaged milk, cream and cottage cheese can be monitored for freshness using polymer indicator. *Dairy and Food Sanitation, 7*, 402–404.

Dainty, R. H. (1996). Chemical/biochemical detection of spoilage. *International Journal of Food Microbiology, 33*, 19–33.

Fu, B., & Labuza, T. P. (1995). Potential use of time–temperature indicators as an indicator of temperature abuse of MAP products. In J. M. Farber, & K. L. Dodds (Eds.), *Principles of modified-atmosphere and sous vide product packaging* (pp. 385–423). USA: Technomic Publishing Company, Inc.

Fu, B., Taoukis, P. S., & Labuza, T. P. (1991). Predictive microbiology for monitoring spoilage of dairy products with time–temperature integrators. *Journal of Food Science, 56*, 1209–1215.

Gram, L., Ravn, L., Rasch, M., Bartholin Bruhn, J., Christensen, A. B., & Givskov, M. (2002). Food spoilage-interactions between food spoilage bacteria. *International Journal of Food Microbiology, 78*, 79–97.

Martley, F. G., Jayashankar, S. R., & Lawrence, R. C. (1970). An improved agar medium for the detection of proteolytic organisms in total bacterial counts. *Journal of Applied Bacteriology, 33*, 363–370.

Rajamäki, T., Alakomi, H.-L., Ritvanen, T., Skyttä, E., Smolander, M., & Ahvenainen, R. *International Journal of Food Microbiology*, submitted.

Riva, M. (1997). Shelf life and temperature prediction in food commercial chain with TTI devices. In: *Proceedings of the 2nd workshop on the ''Developments in the Italian PhD in Food Biotechnology''* Bertinoro.

Russell, N. J. (2002). Bacterial membranes: the effect of chill storage and food processing. An overview. *International Journal of Food Microbiology, 79*, 27–34.

Selman, J. D. (1995). Time–temperature indicators. In M. L. Rooney (Ed.), *Active food packaging* (pp. 213–237). UK: Blackie Academic & Professional.

Senter, S. D., Arnold, J. W., & Chew, V. (2000). APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13 °C and under simulated temperature abuse conditions. *Journal of the Science of Food and Agriculture, 80*, 1559–1564.

Singh, R. P., & Well, J. H. (1987). Monitoring quality changes in stored frozen strawberries with time–temperature indicators. *International Journal of Refrigeration, 10*, 296–300.

Taoukis, P. S., Fu, B., & Labuza, T. P. (1991). Time–temperature indicators. *Food Technology* (October), 70–82.