Available online at
www.ijacskros.com

Indian
Journal of Advances in
Chemical Science

Indian Journal of Advances in Chemical Science S1 (2016) 68-72

Lactic Acid Based Spoilage Indicator for Milk

Mrudula Guggilla, B. Rajeshwar, S. Matche

Department of Food Packaging Technology, CSIR - Central Food Technological Research Institute, Mysore, Karnataka, India.

Received 30th March 2016; Revised 03rd May 2016; Accepted 04th May 2016

ABSTRACT

Food packaging plays a significant role in food product marketing. Attempts have been made to develop the spoilage indicator for milk based on the diffusion of lactic acid, which is temperature dependent. Four lactic-acid based indicators were obtained at different substrate concentrations. An irreversible color change of the pH sensitive indicator (from light green to red) clearly and progressively occurred due to gradual pH reduction; here, the vapor diffusion of lactic acid occurs which indicates the pH reduction. This indicator used as both external and internal indicators for the detection of milk spoilage. This indicator incorporated in the packed milk film with the help of the unidirectional permeable membrane. The unidirectional permeable membrane allows only one direction that is from milk to the indicator and avoids backward flow which prevents the migration into the milk. Time-temperature indicator (TTI) to be considered as good candidates to monitor quality losses of food the activation (Ea) values of TTIs and activation (Ea) values of quality food losses have to match.

Key words: *Time-temperature indicator, Milk shelf life, Shelf life indicator, Smart packaging.*

1. INTRODUCTION

The quality of the food product may be monitored, controlled, and recorded with the help of the modern quality assurance system adaptation [1]. To monitor and record the product quality attributes primarily temperature attribute during the production, storage distribution is quite important [2]. Time-temperature indicators or integrators (TTIs) defined as user-friendly and cost-effective devices to monitor record and communicate the overall effect of temperature history on food product during manufacturing, storage, and distribution [3].

TTI or spoilage indicators response should match the quality losses of food for successful application of indicators to the food products. It is essential that the activation energy of the food products should match with the activation energy of the indicators at the end point of the product shelf life. Hence, the applicability of the spoilage indicator as a quality indicator requires the activation energy studies of both the indicator response and the product (milk) deterioration [4]. Spoilage indicators can be applied to the food products which are sensitive, chilled or frozen food products, meat products, marine products, and dairy products.

A new amylase type TTI was developed based on the reaction between amylase and starch. This type of TTI could be applied to numerous foodstuffs to show the time-temperature history and also to indicate the food quality which deals with the time-temperature exposure [5]. A colorimetric pH-dye based indicator was developed which has the potential as intelligent packaging as a "chemical barcode" for the detection of spoilage of skinless chicken breast. The CO_2 was used as the primary attribute as the degree of deterioration was related to the increased CO_2 and which was more than the total volatile basic nitrogen levels during the storage period [6].

The objectives of this present research were:

1. To study the activation energy of both the indicator response and product deterioration
2. To develop a spoilage indicator for the quality check of the milk
3. To test the indicator as both external and internal indicator for the quality check of milk.

2. EXPERIMENTAL

2.1. Materials and Methods

2.1.1. Materials

Bromothymol blue (HIMEDIA Laboratories, Mumbai) and methyl red (Sarabhai M Chemicals, Baroda) were used as pH dye indicators. Methylcellulose low viscosity (HIMEDIA Laboratories, Mumbai) was used as the mixture dye embedment. Ethanol (Merck Ltd. (I),

*Corresponding Author:
E-mail: rajeshmatche@yahoo.com

Mumbai) and distilled water were used as solvents. Lactic acid (Qualigens Fine Chemicals, Mumbai) was used for the activation of the indicator. Unidirectional permeable membrane for the preparation of indicator model for packed milk.

2.1.2. Preparation of dye mixture

About 50% ethanol was prepared with distilled water. 0.1 g of methyl red was mixed in 50 ml of 50% (v/v) ethanol. Similarly, 0.1 g of bromothymol blue was mixed in 50 ml of ethanol (50%, v/v). These two solutions were taken in the ratio of 1:3 to make the indicator dye mixture.

2.1.3. Preparation of lactic acid based spoilage indicator

About 3 g of methylcellulose was dissolved in 150 ml of distilled water with continuous stirring followed by addition of glycerol as a plasticizer. The solution was stirred till a homogeneous solution was obtained. The solution was filtered in the case of foam formation. The dye mixture prepared was then added to the solution. The solution was spread on Teflon sheet using solvent casting method. This spread solution was kept for drying in the casting room. Film was cut into strips and put into the Petri plates and was activated with a lactic acid solution and tested at different temperatures.

2.1.4. Determination of dynamic parameters of lactic acid based spoilage indicator

The L*, a*, b* chroma system, which uses the corresponding value of the total color difference (TCD) (ΔE) as dynamic parameters, was used to analyze the dynamic change in the indicator's color. The TCD is expressed as following Equation (1):

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

Where, ΔL* is the brightness difference between initiation and each time interval, Δa* is the redness-greenness difference between initiation and each time interval, and Δb* is the yellowness-blueness difference between initiation and each time interval [7].

With the help of 0.25, 0.5, 0.75, and 1 M lactic acid concentration, the Petri plates containing the dye indicator films were tested isothermally at three different temperatures using an incubator (5°C, 26°C, and 37°C). These activated indicators were observed for the color change and with the help of Minolta color analyzer (CM-5 Spectrophotometer, KONICA Minolta, Inc., Osaka) the total color difference was obtained. The instrument used a wide area illumination and a 0° viewing angle, with a 30 mm^2 × 30 mm^2 measuring area. According to the indicator kinetics characterized by Taoukis and Labuza [8], the TCD value X=DE of the indicator could be expressed in terms of a response function as following Equation (2):

$$F(X) = kt \quad (2)$$

Where, k is the reaction rate constant that is correlated with temperature and t is the storage time. By plotting a curve of the response function of TCD F(X) and time, a straight line could be obtained, and the k of different storage temperatures could be calculated from the slope. Taking logarithm on both sides of the Arrhenius function as following Equation (3):

$$\log k = E_a/2.303RT + \log A \quad (3)$$

By plotting a curve between log k and 1/T, a straight line was obtained. The activation energy could be calculated from the slope and A from the intercept directly.

The indicator activated with lactic acid at different temperatures gradually changes the color from light green to red which indicates the final spoilage of the product (milk).

2.1.5. Detection of milk spoilage

Milk packets (Dhodla homogenized milk) were purchased from the Mysore local market. The experiment was setup to find the milk spoilage time with the help of clot-on-boiling (COB) test, and also, pH of the milk was determined at the same interval as of COB test.

2.1.6. COB test

About 5 ml of milk samples of the different temperature range (5°C, 26°C, 37°C) was heated in the test tube to observe the curdling of the milk with 3 h of the time interval.

2.1.7. Testing pH

The pH probe was calibrated using standard reference buffers. A reference buffer of pH 4 and pH 10 was used. The probe was then tested for accuracy in a buffer of pH 7 to confirm the calibration. Every 3 h, the pH of each milk sample was measured and recorded.

3. RESULTS AND DISCUSSION

3.1. pH as a Spoilage Indicator

As it can be observed from Table 1, pH levels of milk at 5°C initially increased, and after 9 h, it gradually decreased with the time. A similar result was obtained at 7°C [9]. This may be due to extensive reformation of hydrophobic bonds does not occur at low temperatures [10,11], which would inhibit reformation of micellar particles and will have buffering action, hence, there was an increase in pH initially. The increase in pH at 5°C up to a certain time and then decreased, this might also be due to the bacterial growth in milk. In general, microbes will be in lag phase until they adapt to the medium, as they adjust the medium they start to increase their cell number which is the log phase leading to the spoilage of milk. As the

bacteria increase in number, the pH of milk will be decreased leading to the milk deterioration [12]. The pH levels of milk samples at 37°C and 26°C decreased gradually with time. This may be due to increasing in solubility of calcium phosphate and more calcium phosphate precipitates, releasing H^+ with a decline in pH with respect to time [13].

The pH of fresh milk should be about 6.7 [14]. It was observed from Table 1; there was a gradual decrease in pH of milk to the critical spoilage point. In the clot on boiling test, all the samples at about 6.28 pH showed positive results showing the curdling of milk. Hence, the pH can be considered as an indicator for the spoilage of milk. At 37°C, milk sample was spoiled at 8 h with a pH of 6.28, whereas at 26°C and 5°C the milk samples were spoiled at 12 h and 72 h with pH of 6.23 and 6.29, respectively.

3.2. Development of Spoilage Indicator for Milk

The activation energies of indicator activated by 0.25, 0.5, 0.75, and 1 M lactic and activation energy of spoilage of milk at three temperatures were calculated. The Petri plates containing the dye indicators of 0.25, 0.5, 0.75, and 1 M lactic acid concentration films were tested isothermally at three different temperatures using an incubator (5°C, 26°C, and 37°C). The change in color of these activated indicators was determined with the help of Minolta color analyzer (CM-5 Spectrophotometer, Konica Minolta, Inc., Osaka) as discussed in materials and methods.

By plotting a curve between ln k and 1/T, a straight line was obtained. The activation energy was calculated from the slope, and A from the intercept directly graphs (Figures 1-5). From the graphs, the slope was obtained and by taking logarithm on both sides of the Arrhenius equation, the activation energy was calculated as it can be seen from Table 2.

Table 1: Measurement of pH of milk and COB test.

Time (h)	pH					
	37°C	COB	26°C	COB	5°C	COB
0	6.7	−ve	6.7	−ve	6.7	−ve
2	6.61	−ve	6.68	−ve	6.88	−ve
4	6.58	−ve	6.63	−ve	6.88	−ve
6	6.49	−ve	6.59	−ve	6.87	−ve
8	6.28	+ve	6.51	−ve	6.84	−ve
10			6.4	−ve	6.81	−ve
12			6.23	−ve	6.78	−ve
24					6.65	−ve
36					6.57	−ve
48					6.48	−ve
72					6.29	+ve

COB=Clot-on-boiling

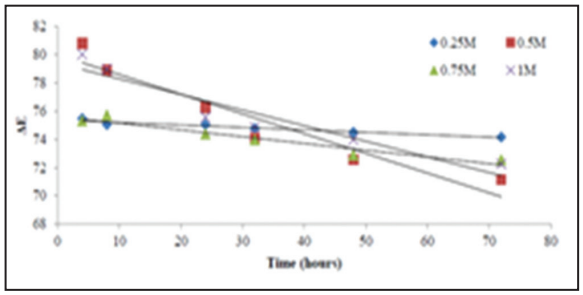

Figure 1: Time versus change in color at 5°C (a) 0.25 M indicator with R^2= 0.9278, (b) 0.5 M indicator with R^2=0.9434, (c) 0.75 M indicator with R^2=0.9267, and (d) 1 M indicator with R^2 =0.9764.

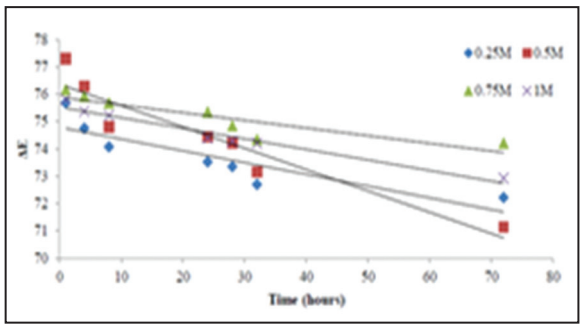

Figure 2: Time versus change in color at 26°C, (a) 0.25 M indicator R2=0.9011, (b) 0.5 M indicator R^2=0.9142, (c) 0.75 M indicator R^2=0.9219, and (d) 1 M indicator R^2=0.9764.

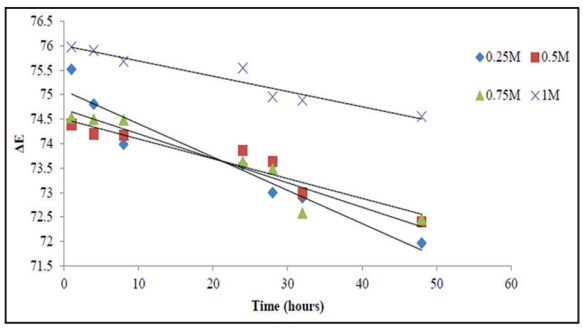

Figure 3: Time versus change in color at 37°C, (a) 0.25 M indicator R^2=0.9285, (b) 0.5 M indicator R^2=0.9125, (c) 0.75 M indicator R^2=0.9187, and (d) 1 M indicator R^2=0.9263.

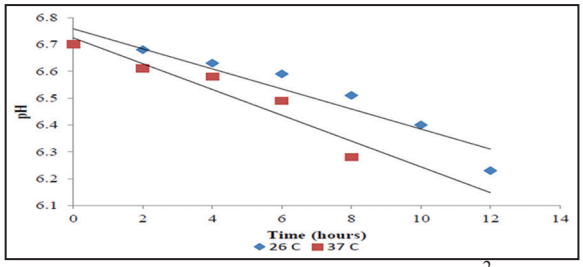

Figure 4: Time versus pH of milk at 26°C, R^2=0.9061 and 37°C R^2=0.9046.

As it can be observed from Table 2, the activation energy of indicator film activated with 0.25 M lactic acid concentration at the 5°C, 26°C, and 37°C temperatures was 21.57, 15.58, and 15.97 kJ/mol, respectively, whereas the activation energy of 0.5 M lactic acid concentration at 5°C, 26°C, and 37°C was 10.43, 12.52, and 19.00 kJ/mol, respectively. Similarly, for 0.75 M lactic acid concentration at 5°C, 26°C, and 37°C temperatures were found to be 16.19, 18.01, and 17.79 kJ/mol, respectively. Activation energy with 1 M lactic acid concentration at 5°C, 26°C, and 37°C temperatures was found to be 11.72, 16.58, and 20.61 kJ/mol, respectively. The activation energy of milk at 5°C, 26°C, and 37°C temperatures was calculated and was found to be 25.71, 18.83, and 18.03 kJ/mol, respectively. Activation energy of the indicator should not be more than the activation energy of spoilage of milk. Both these activation energies should be as close as possible to get the correct indication of the spoilage. From Table 2, can be seen that the activation energy of spoilage of milk is 25.71 kJ/mol at 5°C; the activation energy of 0.75 M is compared to be close. The activation energy of 0.75 M lactic acid indicator at 5°C is 16.19 kJ/mol, at 26°C is 18.01 kJ/mol, and at 37°C is 17.79 kJ/mol. The activation energy of milk at 5°C is 25.71 kJ/mol, at 26°C is 18.83 kJ/mol, and at 37°C is 18.03 kJ/mol. Hence, 0.75 M TTI was a good candidate for the milk spoilage indicator [4,15,16].

The indicator activated with lactic acid at different temperatures gradually changes the color from light green to red which indicates the spoilage of the product (milk). The comparison between the activation energies of indicator activated with different lactic acid concentrations was represented graphically. Here, the activation energy of spoilage indicator activated with 0.75 M was observed to be with 96% confidence level and 3.47% in close range to the range of activation energy of milk spoilage detected using pH measurements (Figure 6).

The indicator can be applied to the milk packaging system both internally and externally. An external indicator, the activation energy of indicator and the milk spoilage, should be matched to be used as an indicator. While the internal indicator can be designed within the package system with the help of the unidirectional permeable membrane and also the migration test was conducted for this particularly designed package film, the results were within specified limits of the standards. With the aid of the unidirectional permeable membrane, only forward direction of the exchange takes place (i.e., it permits the components from milk to indicator and not from indicator to milk), and the membrane avoids the backward flow of the indicator into the milk, thus, the indicator particles won't be migrated into the product. For the migration test 1000 m^2 packaging material about three replicates were taken. Each replicate was exposed to 1000 ml of n-heptane solvent at 38°C for 0.5 h of time. Later, the packing materials were removed, and the extracted solvent was concentrated to 50 ml by distillation. The solvent was then washed and taken into a stainless steel dish and kept for evaporation in an oven at 100±5°C of temperature. After drying, the dishes were cooled in a desiccator for 30 min and weighed; it was observed that the result was within the limits of 60 ppm.

Table 2: Activation energy of milk and indicator film at different temperatures.

Temperature	Activation energy (KJ/Mol)				
	0.25 M	0.5 M	0.75 M	1 M	Milk
5°C	21.57	10.43	16.19	11.72	25.71
26°C	15.58	12.52	18.01	16.58	18.83
37°C	15.97	19.00	17.79	20.61	18.03

When different concentrations of lactic acid were added to the test tube containing dye mixture, a visual change in color of green to red was observed. Bromothymol blue, which shifts from the basic form (blue, pH 7.6) to acidic form (yellow, pH 5.8), results in maximum lambda (λ max) shifting from 615-618 to 430-435 nm. Methyl red, which shifts from the basic form (yellow, pH 6.2) to the acidic form (red, pH 4.5), results in λ max shifting from 430-435 to 523-526 nm [17]. As a mixed dye-based indicator, the light green color changes to red. Wallach [18] reported that a mixed indicator could enhance an expansion of the range of color change as compared with a single indicator.

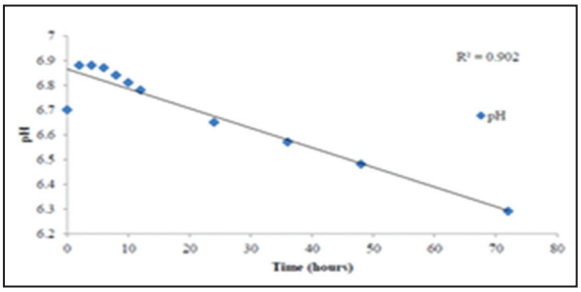

Figure 5: Time versus pH of milk at 5°C.

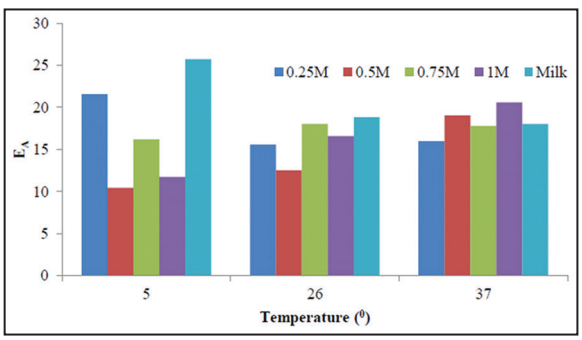

Figure 6: Temperature versus activation energy of 0.25, 0.5, 0.75, and 1 M indicators and milk.

4. CONCLUSION

In this research, the development of lactic acid, spoilage-indicator was based on the lactic acid vapor diffusion. Even though the indicator does not cover up the total food spoilage, the lactic acid-based indicator applies to the dairy products, oxygen sensitive products, and also especially milk. pH detection for the determination of the milk spoilage is a unique and natural method. The irreversible color change of the dye indicator helps in the easy detection of milk/food spoilage. In this study, the use of the unidirectional permeable membrane for designing the packing material was done; further studies should be done for a cheaper source for the utilization of the milk pouches for the designing of the milk spoilage indicator within the milk package system.

5. ACKNOWLEDGMENT

The authors are thankful to Director Central Food Technological Research Institute, Mysore, for the support. The authors acknowledge the help provided by Mr. Prashanth Varaka, Miss. Hafiza Bhanu, and Miss. Sucharitha, for their help and support in this research.

6. REFERENCES

1. K. Koutsoumanis, P. S. Taoukis, G. J. E. Nychas, (2005). Development of safety monitoring and assurance system for chilled food products, ***International Journal of Food Microbiology,*** **100(1-3):** 253-260.
2. P. S. Taoukis, T. P. Labuza, (2003) Time-temperature indicators. In: R. Ahvenainen, (Ed.), ***Novel Food Packaging Techniques,*** Cambridge: Woodhead Publishing Limited, p103-126.
3. M. C. Giannakourou, K. Koutsoumanis, G. J. E. Nychas, P. S. Taoukis, (2005) Field evaluation of the application of time-temperature integrators for monitoring fish quality in the chill chain, ***International Journal of Food Microbiology,*** **102(3):** 323-336.
4. C. Wanihsuksombat, V. Hongtrakul, P. Suppakul, (2010) Development and characterization of a prototype of a lactic acid-based time-temperature indicator for monitoring food product quality, ***Journal of Food Engineering,*** **100:** 427-434.
5. S. Yan, C. Huawei, Z. Limin, R. Fazheng, Z. Luda, Z. Hengtao, (2008) Development and characterization of a new amylase type time–temperature indicator, ***Food Control,*** **19(3):** 315-319.
6. C. Rukchona, A. Nopwinyuwonga, S. Trevanichb, T. Jinkarna, P. Suppakula, (2014) Development of a food spoilage indicator for monitoring freshness of skinless chicken breast, ***Talanta,*** **130:** 547-554.
7. F. J. Francis, (1983) Colorimetry of food. In: M. Peleg, E. B. Bagley, (Eds.), ***Physical Properties of Food***, Westport: AVI Publishing, p105-124.
8. P. S. Taoukis, T. P. Labuza, (1989) Applicability of time–temperature indicators as shelf life monitors of food products**,** ***Journal of Food Science,*** **54(4):** 783-788.
9. M. Zahar, S. R. Tatini, A. Hamara, S. Faushi, (1996) Effect of storage temperature on the keeping quality of commercially pasteurized milk, ***Moroccan Journal of Agricultural and Veterinary Sciences,*** **16(1):** 5-10
10. D. E. Johnston, B. A. Austin, P. M. Murphy, (1992) Effects of high hydrostatic pressure on milk, ***Milchwissenschaft,*** **47:** 760-763.
11. E. C. Needs, R. A. Stenning, A. L, Gill, V. Ferragut, G. T. Rich, (2000) High-pressure treatment of milk: Effects of casein micelle structure and on enzymatic coagulation, ***Journal of Dairy Research,*** **67:** 31-42.
12. T. Y. George, T. Smotzer, (2007) On the lag phase and initial decline of microbial growth curves, ***Journal of Theoretical Biology,*** **244(3):** 511-517.
13. R. Jenness, S. Patton, (1959) ***Principles of Dairy Chemistry,*** New York: John Wiley & Sons, Inc., p176-177.
14. W. Pieter, T. M. Jan, J. G. Wouters, Tom, (2006) ***Dairy Science and Technology,*** 2nd ed. Taylor and Francis: CRC Press, p3-16.
15. W. S. Duyvesteyn, E. Shimoni, T. P. Labuza, (2000) Determination of the end of shelf life for milk using Weibull hazard method, ***Lebensmittel Wissenschaft und Technologie,*** **34:** 143-148.
16. P. S. Taoukis, National Technical University of Athens, (2000) Modelling the use of time-temperature indicators in distribution and stock rotation, ***Food Process Modelling,*** Washington, DC: CRC Press 417.
17. R. W. Sabnis, (2008) ***Handbook of Acid–Base Indicators,*** Boca Raton: CRC Press.
18. D. F. H. Wallach, (1996) Methods and Devices for Detecting Microbial Spoilage in Food Products, US Patent No. 6495368, 2002.

****Bibliographical Sketch***

Miss Mrudula Guggilla is M-Tech Food Processing Technology student of University College of Technology, Osmania University, Hyderabad. Presently, she is doing her project dissertation work in the Department of Food Packaging Technology, CSIR-CFTRI, Mysore. Her research interest's areas are intelligent packaging, active packaging, product development and modified atmospheric packaging of food products.