

Market Survey for Wholesome Lactic Acid Level in Evaporated and Condensed Milk by First Derivative Potentiometric Titration

Alonge, P. O., and *Odokuma-Alonge, O.

Department of Science Laboratory Technology,
Faculty of Life Sciences,
University of Benin,
Benin City,
Edo State,
Nigeria.

*Department of Geology,
Faculty of Physical Sciences,
University of Benin,
Benin City,
Edo State,
Nigeria.

Email: ovie.odokuma-alonge@uniben.edu

Abstract

*Different milk products in markets in Benin City were analyzed for concentration of lactic acid as an index of compliance with permissible values. Nine (09) milk samples coded M₁, M₂, M₃, M₄, M₅, M₆, M₇, M₈ and M₉ were screened and their lactic acid concentration calculated from the equivalent volumes obtained from first derivative potentiometric plots which ranges from 0.36 – 0.51% with a mean value of 0.45%. These values were compared to the approved lactic acid concentration in milk products which is 0.14 - 0.17%. The optimum activities (pKa) of the samples assayed were M₁: 6.06, M₂: 6.22, M₃: 5.98, M₄: 5.93, M₅: 5.94, M₆: 6.07, M₇: 6.35, M₈: 6.23 and M₉: 6.31. This study revealed that all the milk samples in markets in Benin City have values of lactic acid higher than the permissible range. This could be due to exposure to high temperature conditions, deterioration due to activities of *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Streptococcus thermophilus* and poor storage conditions. The results of this study suggest that the regulatory agencies should be proactive in sampling of milk products in the market to avoid epidemics resulting from unwholesome food products. This method can be adopted as a final check procedure for lactic acid content in milk products.*

Keywords: Approved, Lactic Acid, Permissible, pKa, Potentiometric

INTRODUCTION

An intriguing insight into the consumption of milk lies with recently developed biochemical knowledge associated with milk acid content. Milk acid content, which measures the acidity of milk, could be determined by two chemical standpoints: Titratable Acidity (TA) and Hydrogen on concentration (pH). TA is a more reliable indicator because it relates to pH measurement and more sensitive to small changes in milk acidity. The acidity of milk is of two types; natural acidity due to citrates and phosphates present in the milk and dissolved

*Author for Correspondence

CO (carbon monoxide) during the processing of milk, the second is the development of acidity due to lactic acid produced by bacteria using the lactose in the milk as nutrient, converting it to lactic acid (Burke *et al.*, 2018). Proteins (for example, casein alongside amino acids, cysteine and methionine) also influence the acidity of milk (Schmidt *et al.*, 1996). Titratable acidity has since been put in use for many years to indicate the extent to which milk has undergone bacterial degradation (acid production), temperature abuse or is aged; with lactic acid characterized as one of the most suitable chemical compound for the assay and sampling of milk quality.

The acidic activity and titratable lactic acid of processed milk are factors that have impact on human health, which present as the underlying cause of disease condition known as lactic acidosis. Biochemically, an intake or absorption of milk possessing substantial milk acid content (above 0.17%) may affect systemic acid-base homeostasis. A physiological condition, such as prolonged/chronic human acid-base volubility may subsequently result to low grade metabolic acidosis and can become significant in the predisposition to metabolic imbalances such as kidney stone formation, increased bone resorption, reduced bone mineral density, as well as the increased risk of chronic diseases such as type-2 diabetes mellitus, hypertension, and non-alcoholic hepatic steatosis. Also, lactate levels have been shown to predict mortality at a level higher than 5mmol/l (Fall and Szerlip, 2005). This study therefore, is to present lactic acid measurements as a suitable means for the effective monitoring and control of the quality and stability of processed milk including condensed and evaporated milk, full-fat milk, semi-skimmed milk, and skimmed milk by potentiometric acidic measurement of lactic acid present in different grades of milk obtained from different markets within the metropolis of Benin City.

By Principle, the combination of the known concentration of the titrant, the measured volume of titrant addition, and the stoichiometry of the chemical reaction of the titration, allow for accurate determination of the quantity of the corresponding sample constituent that reacts with the chosen titrant. In the case of this method, which depends on simple acid/base titration, the titrant is 0.1 M sodium hydroxide solution, which reacts stoichiometrically with acidic constituents of the sample preparation. For purposes of this method, those acidic constituents are presumed to be lactic acid, and the results of the test are reported as the quantity of lactic acid, on a percentage basis.

The sodium hydroxide used to titrate against lactic acid in this study bears simple stoichiometric relationship with other acids, such as benzoic acid, citric acid and phosphoric acid (He *et al.*, 2018) which are other additives meant for different purposes but particularly useful in the preservation and stabilization of milk in the food market. The results obtained in this study suggests that the processed milk in our markets may have been subjected to high temperature conditions which led to the increase in the biological activities of *Lactobacillus* species (*Lactobacillus bulgaricus*, *Lactobacillus casei* and *Streptococcus thermophiles*) in various milk products thus indicating poor moisture content control which has probably aided in the growth of these bacteria.

METHODOLOGY

Potentiometric Standardization of 0.1M NaOH with 0.1M Sulfamic Acid (H_3NSO_3)

25mL of 0.1M Sulfamic acid solution was pipetted into a 100 mL beaker, to which the glass electrode of the digital pH meter was immersed. The solution in the beaker was potentiometrically titrated against a 50 cm³ solution of 0.1M NaOH solution in a burette. In each case, 1.0 mL of 0.1M NaOH solution was dispensed into the beaker at a time and

electronically stirred. The resultant pH changes and the volume of the solution dispensed were recorded. This procedure was continued until a stable pH reading was recorded.

Potentiometric Standardization of 0.1M NaOH with 0.1M Lactic Acid

25mL of prepared 0.1M Lactic acid solution was pipetted into a 100 mL beaker, to which the glass electrode of the digital pH meter was immersed. The solution in the beaker was potentiometrically titrated against a 50 cm³ solution of 0.1M NaOH solution in a burette. In each case, 1.0 mL of 0.1M NaOH solution was dispensed into the beaker at a time and electronically stirred. The resultant pH change and the volume of the solution dispensed were recorded. This procedure was continued until a stable pH reading was recorded.

Potentiometric Titration of Evaporated Milk Samples with 0.1M NaOH Solution

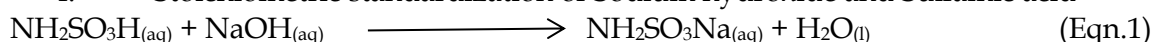
30 mL of the evaporated milk sample was measured into a 100 mL beaker, to which the glass electrode of a digital pH meter was immersed. The solution in the beaker was potentiometrically titrated against a 50 cm³ solution of 0.1M NaOH solution in a burette (0.2 mL of 0.1M NaOH solution was dispensed into the beaker at a time). The solution was electronically stirred. The resultant pH change and the volume of the solution dispensed were recorded. This procedure was continued until a stable pH reading was recorded, signifying the equivalent point of the reaction.

Potentiometric Titration of Powdered Milk Samples with 0.1M Sodium Hydroxide Solution

10 g of the powdered milk sample was weighed into a 100 mL beaker and 30 mL of distilled, deionized water was added to dissolve the powdered sample. The solution was stirred till complete dissolution and the glass electrode of the digital pH meter was immersed. The solution in the beaker was potentiometrically titrated against a 50 cm³ solution of 0.1M NaOH solution in a burette. In each case, 0.2 mL of 0.1M NaOH solution was dispensed into the beaker at a time and electronically stirred. The resultant pH changes and the volume of the solution dispensed were recorded. This procedure was continued until a stable pH reading was recorded.

Calculations

I. Stoichiometric standardization of Sodium hydroxide and Sulfamic acid



$$97.09 \text{ g NH}_2\text{SO}_3 \equiv 1000\text{mL 1M NaOH solution} \quad (\text{Eqn. 2})$$

$$9.709 \text{ g NH}_2\text{SO}_3 \equiv 1000\text{mL 0.1M NaOH solution} \quad (\text{Eqn. 3})$$

$$2.4273\text{g NH}_2\text{SO}_3 \equiv 25 \text{ mL 0.1M NaOH solution} \quad (\text{Eqn. 4})$$

$$0.09709\text{g NH}_2\text{SO}_3\text{H} \equiv 1 \text{ mL 1M NaOH solution} \quad (\text{Eqn. 5})$$

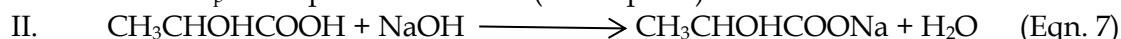
$$\text{Therefore } 2.4273\text{g NH}_2\text{SO}_3\text{H} = \frac{2.4273}{0.09709} = 25 \text{ mL NaOH}$$

Burette reading gave a reading of 25.56 mL

$$\text{Hence, factor, } F_1 = \frac{V_T}{V_P} = \frac{25}{25.56} = 0.9779. \quad (\text{Eqn. 6})$$

where V_T is the theoretical volume (or endpoint) of 0.1M NaOH

V_P is the practical volume (or endpoint) of 0.1M NaOH



$$\text{CH}_3\text{CHOHCOOH} \equiv 1000\text{mL 1M NaOH solution} \quad (\text{Eqn. 8})$$

$$7.597 \text{ mL CH}_3\text{CHOHCOOH} \equiv 1000 \text{ mL 1M NaOH} \quad (\text{Eqn. 9})$$

$$0.007597 \text{ mL CH}_3\text{CHOHCOOH} \equiv 1 \text{ mL 1M NaOH} \quad (\text{Eqn. 10})$$

The stoichiometric relationship between sodium hydroxide and lactic acid ($\text{CH}_3\text{CHOHCOOH}$) is given by equation 9, thus:

$$F_1 \times V_1 = F_2 \times V_2$$

Where, F_1 is the factor of 0.1N NaOH solution

V_1 is the equivalent volume of the 0.1M NaOH solution

F_2 is the factor of 0.1M Lactic acid solution

V_2 is the equivalent volume of the 0.1M lactic acid solution

Hence, $0.9779 \times 25.56 \text{ mL} = F_2 \times 23.00 \text{ mL}$

$$F_2 = 1.08.$$

III. To determine the concentrations of lactic acid (g/ 100mL) in the milk samples coded M_1 - M_9 , the formula below was applied:

$$\text{Lactic acid concentration} = (\text{Equivalent vol.} \times F_2 \times 0.007597) \times 3.33 \quad (\text{Eqn.11})$$

(Acid/Base Titration)

RESULTS AND DISCUSSION

The results of the potentiometric titration of the samples are presented in Figures 1-11 and Table 1, respectively. Lactic acid in the various milk samples such as liquid, powdered and condensed milk types, coded M_1 - M_9 were titrated against standardized 0.10 M sodium hydroxide. Figure 1 shows first derivative potentiometric plot for the standardization of 0.10M sodium hydroxide with sulphamic acid. The factor, F_1 , obtained in this stoichiometric reaction was 0.9779. The 0.10 M standardized sodium hydroxide was titrated potentiometrically with standard lactic acid (coded M_0) of high purity. The calculated factor for lactic acid standardization was 1.08. The standardized lactic acid with a factor ($F_2 = 1.08$) used to assay the lactic acid concentrations in various samples (M_1 - M_9) in this study.

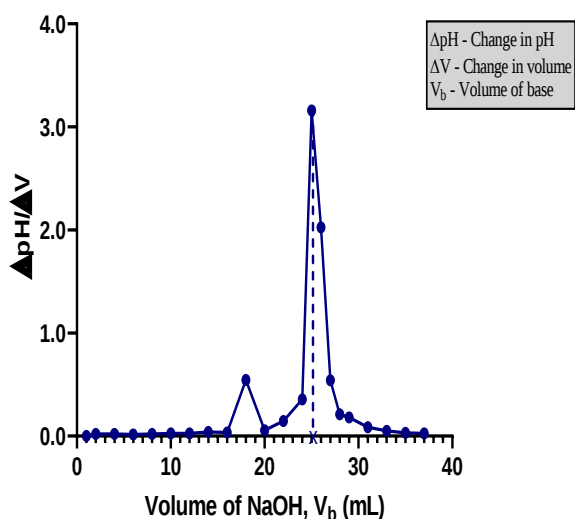


Figure 1: Potentiometric titration plot for the standardization of 0.1M Sodium hydroxide (NaOH) with sulfamic acid as a primary standard

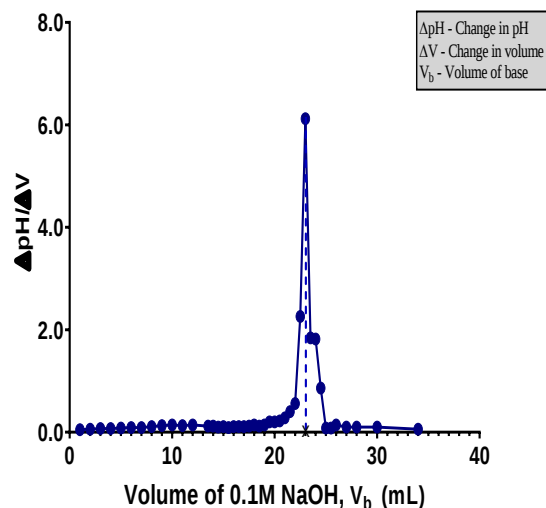


Figure 2: Potentiometric titration plot of standard 0.1M Lactic Acid versus 0.1M Sodium hydroxide, NaOH solution.

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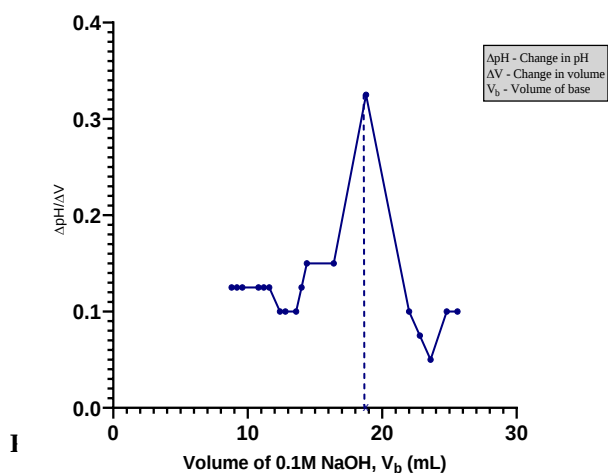


Figure 3: Potentiometric titration plot for the determination of the concentration of lactic acid in sample M_1 titrated against 0.1M NaOH solution.

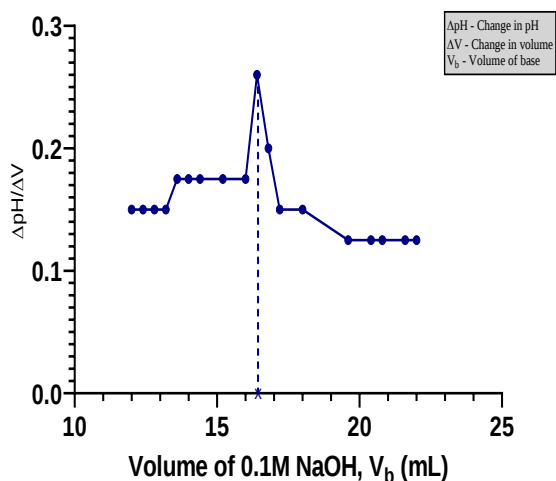


Figure 4: Potentiometric titration plot for the determination of the concentration of Lactic Acid in sample M_2 titrated against 0.1M NaOH solution.

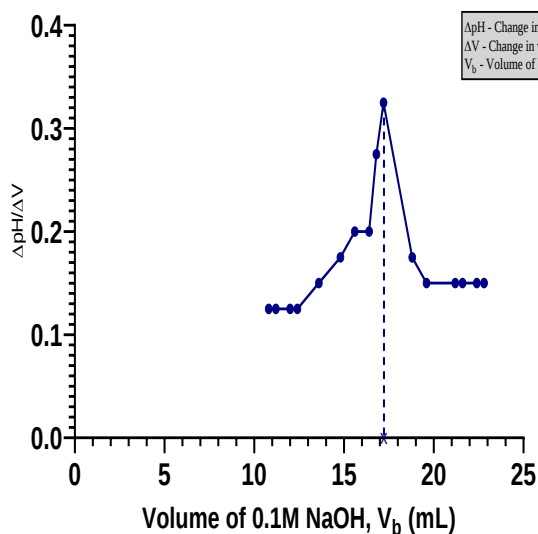


Figure 5: Potentiometric titration plot for the determination of the concentration of Lactic acid in sample M_3 titrated against 0.1M NaOH solution.

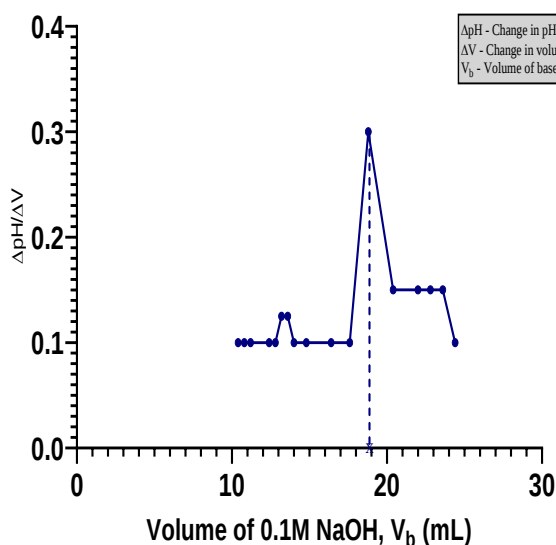


Figure 6: Potentiometric titration plot for the determination of the concentration of Lactic acid in sample M_4 titrated against 0.1M NaOH solution

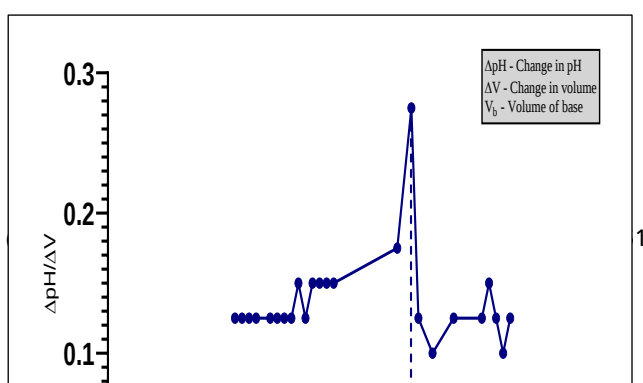
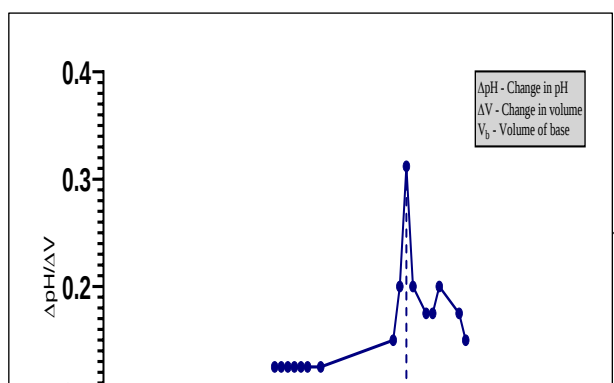


Figure 7: Potentiometric titration plot for the determination of the concentration of Lactic acid in sample M₅ titrated against 0.1M NaOH solution.

Figure 8: Potentiometric titration plot for the determination of the concentration of Lactic acid in sample M₆ titrated against 0.1M NaOH solution.

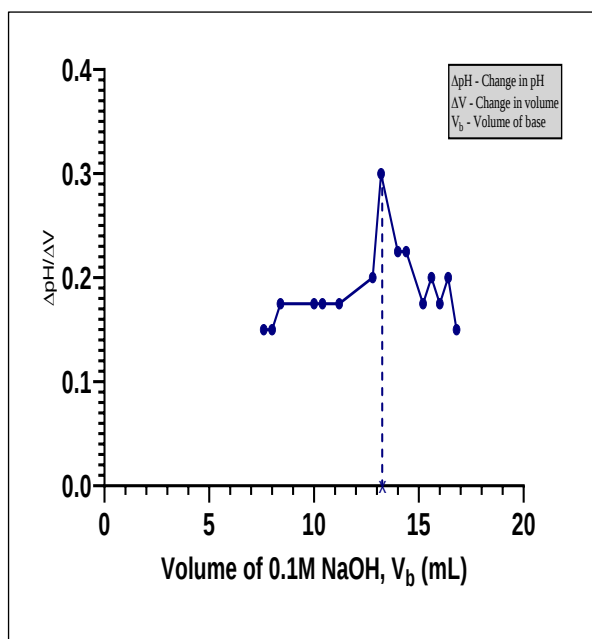
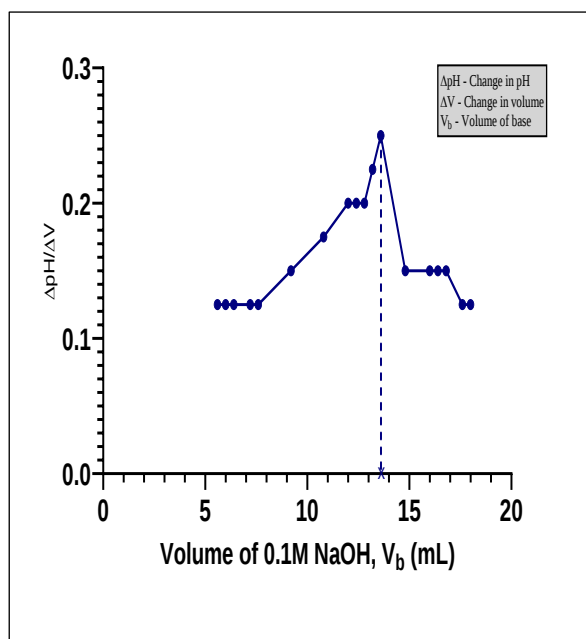


Figure 9: Potentiometric titration plot for the determination of the concentration of Lactic Acid in sample M₇ titrated against 0.1M NaOH solution.

Figure 10: Potentiometric titration plot for the determination of the concentration of Lactic Acid in sample M₈ titrated against 0.1M NaOH solution. The equivalent volume of 0.1M NaOH was 13.20 ml.

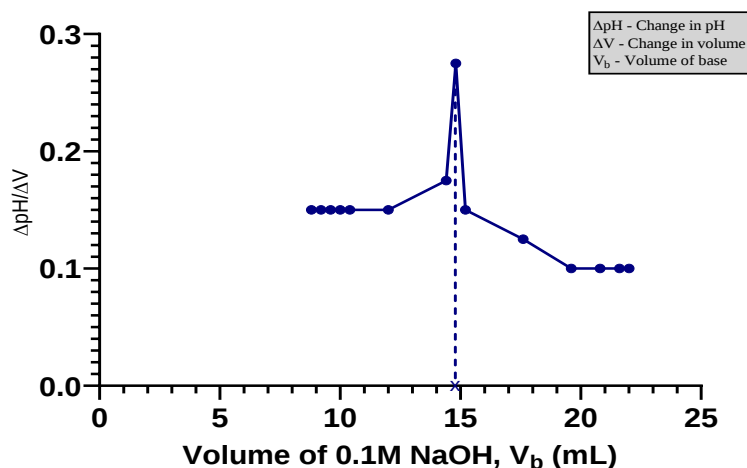


Figure 11: Potentiometric titration plot for the determination of the concentration of lactic acid in sample M₉ titrated against 0.1M NaOH solution.

Table 1: Summary of the various parameters screened and analyzed during the potentiometric titration of milk samples M₁-M₉.

Samples	Factor of NaOH (F ₁)	Factor of Lactic Acid (F ₂)	Equivalent pH	Equivalent Volume (mL)	Concentration of Lactic Acid (g/100 mL of milk)*	Standard permissible levels of lactic acid concentration (g/100 mL of milk)**	pKa
M ₁	0.9779	1.08	7.67	18.80	0.5137	0.14-0.17	6.06
M ₂	0.9779	1.08	7.49	16.60	0.4535	0.14-0.17	6.22
M ₃	0.9779	1.08	7.47	17.20	0.4669	0.14-0.17	5.98
M ₄	0.9779	1.08	7.15	18.60	0.5082	0.14-0.17	5.93
M ₅	0.9779	1.08	7.40	18.40	0.5025	0.14-0.17	5.94
M ₆	0.9779	1.08	7.63	17.20	0.4699	0.14-0.17	6.07
M ₇	0.9779	1.08	7.60	13.60	0.3716	0.14-0.17	6.35
M ₈	0.9779	1.08	7.56	13.20	0.3606	0.14-0.17	6.23
M ₉	0.9779	1.08	7.63	14.80	0.4043	0.14-0.17	6.31

* The concentration of lactic acid determined from sample M₁-M₉ by potentiometric titration ranged from 0.36-0.51g/100mL of the milk samples screened.

** The standard permissible levels of lactic acid concentrations for milk products as stated by the US Department of Health and Human Services (1993).

In Figure 3, the lactic acid level in sample M₁ was determined potentiometrically using a calibrated pH meter. The equivalent pH and equivalent volume obtained from this plot are 7.67 and 18.80 mL respectively. The concentration of lactic acid calculated was 0.5137g/100 mL of the milk sample. This value was found to be higher than the standard 0.14 - 0.17% (expressed as lactic acid), permissible by regulatory agencies (Schmidt *et al.*, 1996). The pKa value obtained in this reaction was 6.06.

Figure 4, shows the first derivative potentiometric curve for the titration of sample M₂ against 0.1M NaOH solution. The equivalent pH and equivalent volume obtained were 7.49 and 16.60 mL respectively and 100 mL of the milk sample, coded M₂ was found to contain 0.4535g of lactic acid and the pKa of the reaction was 6.22. The concentration of lactic acid in the milk

sample exceeds the specified range as indicated by Schmidt *et al.*, (1996). This indicates unwholesomeness in the milk product.

The equivalence point is found in figure 5 as the maximum point in the plot. From the plot, the equivalent pH and equivalent volume obtained for the milk sample coded M₃, are 7.47 and 17.20 mL respectively. The amount of lactic acid per 100 mL of the milk sample coded M₃ was found to be 0.4669g and the activity of the acid in the reaction, indicated as the pKa, was observed at 5.98. The pKa consequent to the total acid content (expressed as lactic acid) in sample M₃, was of moderate acidic strength and acid dissociation.

The equivalent pH and equivalent volume obtained from the plot in Figure 6 are 7.15 and 18.60 mL respectively. The method of potentiometric titration employed gave the calculated concentration of lactic acid in the milk sample coded M₄ as 0.5082g/100 mL of the milk sample coded M₄. This show a deviation from the normal range of permissible lactic acid levels in milk products as given by the U.S. Department of Health and Human Services (1993). The pKa value obtained in this reaction was 5.93. The concentration of lactic acid in milk sample M₄ indicates unwholesomeness of the milk product in comparison to the already set regulatory standards.

In Figure 7, the lactic acid level in sample M₅ was determined potentiometrically by titration against standardized 0.1M NaOH solution via direct responses of the glass electrode of the calibrated pH meter to pH changes. The equivalent pH and equivalent volume obtained are 7.40 and 18.40mL, respectively. The concentration of lactic acid present was found to be 0.5025g/100mL of the milk sample coded M₅. In Figure 7, the pH change per unit change in volume, are in direct relationship with acidity changes. Hence, the acidity constant, pKa of sample M₅ was observed to be 5.94.

Figure 8 shows the points of equivalent pH and equivalent volume of milk sample M₆ as 7.63 and 17.20 mL respectively. The concentration of lactic acid in the milk sample was determined as 0.4699g/100 mL of milk sample. The pKa however, of the sample was found to be 6.07.

In Figure 9, the screened milk sample M₇ was titrated potentiometrically against 0.1M NaOH during the determination for lactic acid content of the sample. The equivalent pH and equivalent volume obtained were 7.60 and 13.60 mL respectively. 100 mL of the milk sample was found to contain 0.3716g lactic acid and the pKa of the reaction was 6.22. The lactic acid concentration of the milk sample (M₇) observed exceeded permissible levels; however, the concentration amount was lower when related to milk samples M₁-M₆. The pKa of the reaction was observed at a value of 6.35.

Figure 10 shows the potentiometric titration of sample M₈ against 0.1M NaOH solution. The equivalent pH and equivalent volume obtained from this plot are 7.56 and 13.20 mL respectively. The concentration of lactic acid calculated in the milk sample coded M₈was 0.3606g/100 mL. This value is regarded as unwholesome in comparison with the standard values given by food regulatory and governing agencies. The pKa value obtained in this reaction was 6.23.

In figure 11, the equivalent pH and equivalent volume obtained from the potentiometric titration of the milk sample M₉ against 0.1M NaOH are 7.63 and 14.80 mL respectively. The determined concentration of lactic acid present within the milk sample coded M₉, was 0.4043g/100 mL of the milk sample and the strength of the acid in the reaction, indicated as

the pKa, was observed at 6.31. The pKa (expressed as lactic acid) of sample M₉, was of moderate acidic strength and acid dissociation.

Lactic acidosis has been established by Works in literature to occur at lactic levels exceeding 5mmol/L (or 0.45g/L) (Fall and Szerlip, 2005). Lactic acidosis is generally defined as a serum lactate concentration above 4 mmol/L, which is often accompanied by a blood pH of less than 7.35 and a plasma bicarbonate concentration below 20 mmol/L. These values are commonly used in clinical practice; however, the absolute thresholds may vary depending on coexisting respiratory or metabolic acid-base disorders, which can partially mask or offset the expected acid-base changes. In contrast, hyperlactatemia refers to lactate levels above 2 mmol/L without associated acidosis. (Kamel *et. al.*, 2020). Lactic acidosis is the most common cause of metabolic acidosis in hospitalized patients. Although it is typically associated with an elevated anion gap, moderately increased lactate levels can occur with a normal anion gap, particularly in the presence of hypoalbuminemia, unless the gap is appropriately corrected. (Zanza *et. al.*, 2022). However, lactic acid concentration values obtained from this study range from 3.60-5.10 g/L in all the milk samples (M₁-M₉) assayed. This suggests that the consumption of these milk samples assayed could lead to the incidence of lactic acidosis, particularly in underage children.

CONCLUSION

Critical examinations of the results obtained in this study reveals unwholesomeness which might have resulted from inappropriate manufacturing practices and poor storage conditions of all the milk samples assayed in this study. The results obtained in this study suggests that the processed milk in our markets may have been subjected to high temperature conditions which led to the increase in the biological activities of *Lactobacillus* species (*Lactobacillus bulgaricus*, *Lactobacillus casei* and *Streptococcus thermophiles*) in various milk products thus indicating poor moisture content control which has probably aided in the growth of these bacteria.

As community service function of the university, this appears to be potent information that could lead to proper manufacturing practices of milk products in Nigeria. The potentiometric titration adopted in this study could also be a final check method that can be adopted by food and drug regulatory agencies in Nigeria in the determination of adherence of milk processing industries to permissible levels of acid content in milk through adequate quality control mechanism.

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