**Biotechnology lab: pH, lactic acid and Dornic degree**

The quality of a cheese depends mainly on the quality of the raw material, milk. Milk is a highly perishable product. The freshness of milk can be determined by measuring its acidity. The acidity of milk is measured in Dornic degrees (°D): 1 Dornic degree corresponds to 0.1 g of lactic acid per litre of milk.

Fresh milk has an acidity of 15 to 18 °D and contains approximately 5% lactose. The bacteria present in the milk will transform the lactose into lactic acid by the process of fermentation, which will modify the pH of the milk and increase the Dornic degree.

When the acidity exceeds 37°D, the casein will flocculate (curdle). The less fresh the milk is, the more bacterial activity increases and the greater its total acidity.

Determining the acidity of a milk is therefore a simple way to determine the freshness of the milk.

**Objective**

Determine the freshness of milk by determining the lactic acid concentration in milk. Express the result in Dornic degrees.

Determine the lactic acid concentration of transformed milk (fresh cheese or plain yogurt)

**Material**

- 25 mL burette

- Burette holder

- Universal holder

- 20 mL fresh milk

- 20 mL fresh cheese or plain yogurt

- Sodium hydroxide solution 0.05 mol/L and 0.25 mol/L

- pH meter

- Distilled water

- Phenolphthalein (1% solution in 95% ethanol)

- Bromothymol blue

- 25 mL or 10 mL volumetric pipette and a sampling device

- Magnetic stirrer and its magnetic bar

- Graduated cylinder

- Beaker labelled “waste”

- 2 beakers or Erlenmeyer flasks labelled “milk” and “cheese”

**Method**

**Part 1 — Titration of lactic acid in milk**

1. **Fill** the burette with the 0.05 mol/L sodium hydroxide solution.
2. **Adjust** the liquid level to zero in the burette by pouring the overflow into the “waste” beaker.
3. Using the volumetric pipette**, take** 20 mL of milk and **pour** it into the beaker identified as “milk”
4. **Add** 10 drops of phenolphthalein.
5. **Insert** the bar magnet.
6. **Place** the beaker under the burette on the stirrer.
7. **Adjust** the stirrer to ensure that the mixture in the beaker does not splash to the sides.
8. **Place** the pH meter in the milk.
9. **Pour** in the sodium hydroxide solution mL by mL and measure the pH after each mL is added.
10. **Locate** the equivalence point. When the solution in the Erlenmeyer flask changes colour persistently where the neutralizing solution comes into contact with the solution to be neutralized.

**Observation**

1. Compile the data into a table.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Vb (mL) | **0.0** | **1.0** | **2.0** | **3.0** | **4.0** | **5.0** | **6.0** | **7.0** | **8.0** | **9.0** | **10.0** | **11.0** | **12.0** | **13.0** | **14.0** | **15.0** |
| pH |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

1. Draw the pH-metric titration curve: pH = f (Vb), Vb being the volume of sodium hydroxide solution added. Determine the volume poured at the equivalence.
2. Place on the curve the point corresponding to the appearance of the colour of the coloured indicator. Show that this colour indicator is suitable for locating the equivalence in a titration using a colour indicator.

**Part 2 — Titration of lactic acid in fresh cheese or plain yogurt**

1. **Fill** the burette with the 0.25 mol/L sodium hydroxide solution.
2. **Adjust** the liquid level to zero in the burette by pouring the overflow into the “waste” beaker.
3. **Measure out** 20 mL of fresh cheese or yogurt.
4. **Stir** the fresh cheese or yogurt in the beaker marked “cheese” until liquid. Add a little distilled water if necessary if the product is too firm.
5. **Add** distilled water to obtain 75 mL of mixture.
6. **Add** 10 drops of bromothymol blue.
7. **Insert** the magnet bar.
8. **Place** the beaker under the burette on the shaker.
9. **Adjust** the stirrer to ensure that the mixture in the beaker does not splash to the sides.
10. **Place** the pH meter in the milk.
11. **Add** the sodium hydroxide solution mL by mL. When the solution in the beaker begins to change colour, add the sodium hydroxide solution drop by drop.
12. Continue **pouring drop by drop** until the solution in the Erlenmeyer flask persistently changes colour where the neutralizing solution comes into contact with the solution to be neutralized.
13. **Record** the value of the equivalent volume in mL.

**Analysis**

1. Represent the structural formula of lactic acid. Identify the different characteristic groups present.
2. Write the chemical reaction of lactic acid and sodium hydroxide.
3. Determine the molar concentration of the acid in the milk, based on the data and the result of the experiment and indicate the Dornic degree of the milk used. Is this milk fresh? The molar mass of lactic acid is given: M = 90 mol/L.
4. Determine the Dornic degree of the fresh cheese or yogurt.
5. A group of students performed a titration of an unknown milk following the protocol used. The values of equivalence volumes obtained are:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | 1 | 2 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Veq/mL | 13.0 | 13.0 | 10.0 | 10.0 | 12.7 | 11.2 | 11.2 | 12.6 | 11.2 |

 Is this milk safe to drink? Explain your answer.

1. Compare the Dornic degrees of milk and cheese or yogurt.

**Note:** use the following formula to calculate the Dornic degree:

 $°D= \left[NaOH\right]\*Veq\*90\*10 $

 20