Development of On-Site Measurement of Calcium in Food Samples by Use of Ion Selective Electrode

example for Using <LAQUAtwin> Ca^{2+} Ion meter

Hisashi YAMANOUCHI

The concentration of calcium in food samples is commonly measured by use of Inductively Coupled Plasma (ICP), Ion Chromatography (IC) and Atomic Absorption Spectrophotometry (AAS). However, these methods are not appropriate for on-site measurement at production lines and farms because these instruments are too large to carry out. In this paper, we propose new measurement method which dissociates the compound including calcium into the calcium ion adjusting pH of samples before the potentiometric measurement with the compact calcium ion meter (abbreviated as Ca^{2+}-meter). The results obtained by use of Ca^{2+}-meter with pretreatment were good agreement with that obtained by use of ICP. We can measure all amount of calcium in milk products by use of Ca^{2+}-meter with pretreatment which generate free calcium ions from the calcium compound in milk products.

Introduction

Calcium is one of the most important elements for our body. As a major mineralization of bone and teeth, calcium constitute a few percent of the body's weight. It has an important role as a signal for many cellular processes such as nerves work and muscle movement. We can take it into our body by eating which milk products and fish have much calcium..

The concentration of calcium in food samples is commonly measured by use of Inductively Coupled Plasma (ICP), Ion Chromatography (IC) and Atomic Absorption Spectrophotometry (AAS).[1] However, these methods are not appropriate for on-site measurement at production lines and farms because these instruments are too large to carry out and they need for complicated pretreatment. Recently, for on-site measurement of calcium, the minimization and simplification of instrument is demanded. An ion selective electrode is one of the suitable sensors for the on-site measurement of calcium.

A calcium ion selective electrode (abbreviated as Ca^{2+}-ISE) consists of a working electrode and a reference electrode. The cell voltage between two electrodes varies depending on the concentration of calcium ion in the solution. The potentiometric measurement by use of Ca^{2+}-ISE is cheaper and smaller than that used for above mentioned methods. However the Ca^{2+}-ISE is not sensitive to calcium binding protein and various organic acids in food because an ion selective electrode can respond only dissociated ions in aqueous solution. Accordingly, when we determine total amount of calcium in food by use of

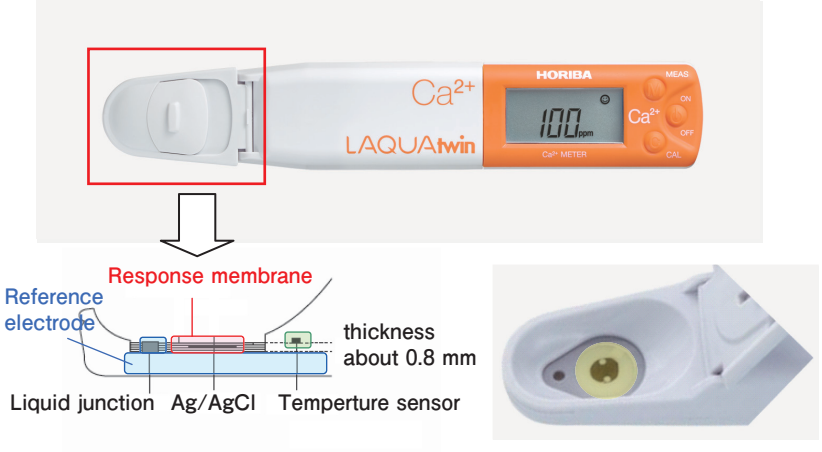

Figure 1 Illustration of the calcium ion meter (B-751)

Ca^{2+}-ISE, the special pretreatment which makes the calcium in the compound into a free calcium ion is needed.

In this paper, we propose new measurement method which dissociates the compound including calcium into the calcium ion adjusting pH of samples before the potentiometric measurement with Ca^{2+}-ISE. By use of this method, we can easily measure the concentration of calcium and the measured values correlate well with that obtained by use of ICP. In addition, we introduce the compact calcium ion meter (abbreviated as Ca^{2+}-meter, Horiba, Ltd, LAQUAtwin B-751) which newly released in September 2012.

Experimental

Sample.

Milk products contained much calcium were prepared. Two milks and two lactic acid were kept at room temperature (22~24 °C) during the measurements.

Instrument and standard solution.

Figure 1 illustrates the structure of Ca^{2+}-meter which consists of the calcium ion sensor, the potentiometer and the display. The ion selective membrane and liquid junction of reference electrode were located on the same plane surface whose thickness was about 0.8 mm. This instrument enables us to measure small volume sample (about 300µL).

The ion meter was calibrated with two standard solutions before measurements. The 150 ppm and 2000 ppm calcium standard solutions (3.74 mmol dm^{-3} and 49.9 mmol dm^{-3} at $CaCl_2$) were used for the two point calibration. 0.1 mol dm^{-3} KCl was added into the standard solutions as supporting electrolyte. The intercept (zero point) and slope for the calibration curve of the cell voltage against the concentration of calcium ion were determined from two point calibrations. Compared with the Ca^{2+}-meter, the concentration of calcium in same samples was measured with ICP (Horiba, Ltd., ACTIV-M ICP).

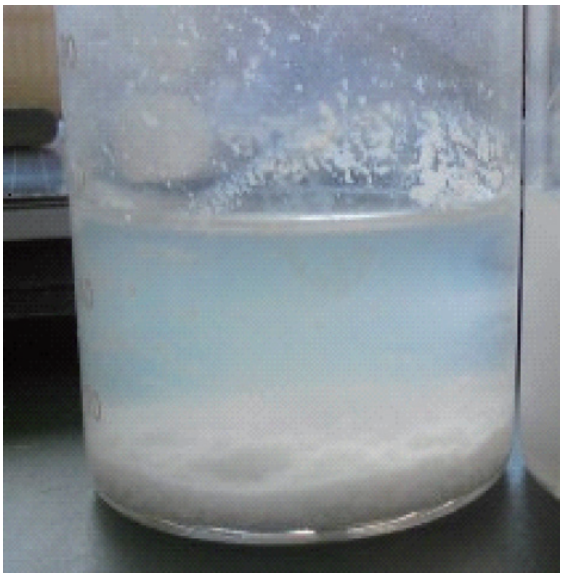

Figure 2 Illustration of milk product sample after pretreatment

Pretreatment of sample

Milk products contain calcium bonding casein protein and lactic acid compounds. The pH of these sample was controlled to 2 with adding strong acid in order to break these bonds, and then it was adjusted to among 4.3 to 4.6 by diluting with ion-exchange water and adding appropriate amount of Tris powder. There are two reasons why we control pH in sample. First, the interference of hydrogen ions causes the measurement errors.[2] Second, the free calcium ion dissociated from calcium compound may recombine casein protein and lactic acid when the pH value in sample is more than 4.6.

Supernatant liquid of samples after pretreatment was measured. The details of pretreatment procedures are as follows.

Pretreatment procedures

1. Preparation of 5 mL sample.
2. Adding of 100µL of 5 mol dm^{-3} HCl to sample.
3. Dilution by adding ion exchange water to 50 mL.
4. Additoin of 0.05 g of Tris (hydroxymethyl) aminomethane to diluting sample to control pH4.3~4.6.
5. Keeping on leaving diluting sample for a few minutes. (Figure 2) The casein protein precipitated in pH 4.3~4.6 because the isoelectric point of casein is 4.6.
6. Removing 5 mL of supernatant liquid from diluting sample.
7. Dissolving 0.0375 g of KCl in 5 mL of supernatant liquid.

The concentration of calcium in samples was calculated by multiplying experimental values obtained by use of Ca^{2+}-meter by ten because the tenfold dilution was made of the sample through the pretreatment. In the case of the measurement with ICP, the thousandfold dilution was made of sample by ion exchange water.

Results and Discussion

Figure 3 shows the difference cell voltage (ΔE_{pH}) dependence on pH with ion meter at 1 mmol dm^{-3} $CaCl_2$ and 0.1 mol dm^{-3} KCl. The difference between E values at

other pH $CaCl_2$ solution except pH 7.5, $E_{pH\,(X)}$ and at pH 7.5 $CaCl_2$ solution, $E_{pH7.5}$, $\Delta E_{pH} = E_{pH\,(X)} - E_{pH\,7.5}$, is given in Figure 3. The measurement of E at each pH was repeated with three different ion meters. Experimental values were obtained from the average of E values and the range of error bar shows the difference between maximum and minimum values for three measurements. The pH of each solution was controlled to among 2 to 12 with adding HCl or NaOH.

The ΔE_{pH} value was independent on pH in the range of pH 5~12. The ΔE_{pH} value changed due to the interference of hydrogen ion on the Ca^{2+}-ISE in a solution whose pH was less than 4. The change of pH from 4 to 2 in sample decrease the cell voltage by about 60 mV which corresponds to the 1/100 dilution of the concentration of calcium ion. Therefore, pH of samples must be controlled above 4 to prevent the interference of pH on the Ca^{2+}-ISE.

Figuer 4 shows comparison of experimental values obtained by use of Ca^{2+}-meter and ICP at four milk products. Measurements by use of Ca^{2+}-meter by with pretreatment (□) and without pretreatment (○) were carried out to confirm the effect of pretreatment on measurement of calcium. The results obtained by use of Ca^{2+}-meter with pretreatment were good agreement with that obtained by use of ICP. The correlation coefficient, r^2 was 0.952. Experimental values obtained by use of Ca^{2+}-meter without pretreatment were less than a half of that obtained with the pretreatment.

Thus, we can measure all amount of calcium in milk products by use of Ca^{2+}-meter with pretreatment which generate free calcium ions from the calcium compound in milk products.

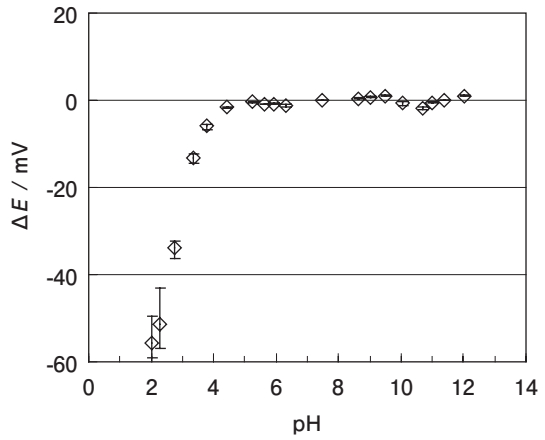

Figure 3 Dependence of voltages generated by the calcium ion meter on the hydrogen ion activity.

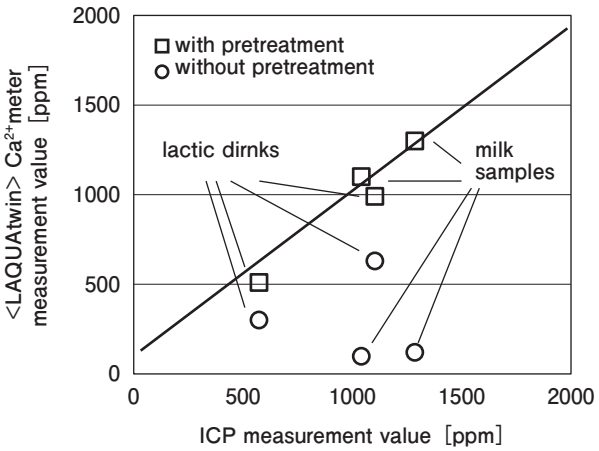

Figure 4 Relationship between measurement value using by calciumion meter and ICP. Four samples were measured with pretreatment (◇) and without pretreatment (□).

Conclusion

By use of Ca^{2+}-meter with pretreatment, calcium concentration in milk products were determined. This method enables us to measure more easily total amount of calcium in milk products on-site at production lines and farms than the expensive instrument such as ICP, IC and AAS. It is expected that this method will be widely used on-site measurement.

On-site measurement of calcium will be more required in the food industry because of the increase of interest in healthy, safety and peace of mind. We expect this method can be helpful in these demands.

References

[1] JIS K 0400-50-20, JIS K 0400-52-30
[2] J. RUZICKA, E. H. HANSEN, J. CHR, TJELL, 160-173

Hisashi YAMANOUCHI
Liquid and Water Quality R&D Dept.
Application R&D Center
Reserch & Development Division
HORIBA, Ltd.