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Study of a Rapid Detection Method for Protein Content in Milk and Development of Detection Equipment

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Abstract

Among the nutrients in milk, protein content is a core indicator for measuring the quality of milk. Therefore, to explore a rapid detection method for milk protein content and develop rapid detection equipment for protein ingredient can provide reference for quality analysis and quality control of the production process. In this paper, different dairy protein detection methods are analyzed, the working principles of Micro-Kjeldahl method are studied, and a new type of milk protein content detector is designed based on the Micro-Kjeldahl nitrogen determination method. As for this instrument, LPC2214 microcontroller with ARM structure is taken as the control core, with the functions such as adding certain volume of lye, adding certain volume of boric acid absorption liquid, automatically generating steam required, automatically titrating hydrochloric acid solution, accurately determining the endpoint, conveniently setting parameters and so on, which realizes the digital and intelligent design in determining the protein content in dairy products. Experimental results show that the system is stable, with broad application prospects.

Keywords: *Micro-Kjeldahl method; protein detection; embedded system; photoelectric detection*

1. Introduction

Milk and its products have high nutritional value. With the increase of people's health awareness, the role of dairy products with rich nutrients becomes increasingly important in daily life. Among all the nutrients in milk, protein content is a core indicator for measuring the quality of milk. The measurement of protein content in dairy products gains much concern, especially the rapid detection method for large volume. To explore a rapid detection method for milk protein content and develop rapid detection equipment for protein ingredient can provide reference for quality analysis and quality control of the production process. Micro-Kjeldahl method is taken as the theoretical basis in this paper, and a new type of milk protein content detector is designed by analyzing the principle of Micro-Kjeldahl nitrogen determination method. The overall structure and the detection process system are set up in the paper; the system adopts the LPC2214 microcontroller with ARM architecture from PHILIPS company as the control core, to realize full automation of milk protein content; the operator only needs to put the sample into the instrument, to get accurate results soon.

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2. Rapid Detection Method of Protein and the Development of Detection Equipment

At present, there are multiple measurement methods for the dairy protein composition testing, in which the more common methods include chemical method, ultrasonic analysis and infrared spectroscopy.

Chemical method is a traditional method, of which the result accuracy is the highest, and all other measurement methods should be closer to the chemical method. Although chemical method is mature, with high precision, detection time is long, many reagents are required, testing cost is high, and skilled persons are required, so only timed and quantitative sampling detection can be conducted, rather than real-time online testing, and dynamic quality control cannot be realized for the dairy production process as well.

Ultrasonic analysis method is to make use of the interactions between high-frequency sound waves and substances in order to obtain the internal physical and chemical properties of the tested substance. Currently, the prediction accuracy value by ultrasonic method to detect the milk components can reach the level of 5% [1].

Infrared spectroscopy is a modern analytical technique, which involves spectroscopic measurement techniques, chemical metrology, computer technology and basic measurement. Infrared spectroscopy analysis has the features of low cost, high speed, easy operation, not destroying the location of measured samples, and not using chemical reagents. But infrared spectroscopy products are expensive, with larger instrument volume, which need stringent experimental conditions, not suitable for field operation [2].

Micro-Kjeldahl method is the most common method for the determination of protein, which is one of the most accurate methods for the determination of the protein content in milk products, and taken as the legal standard test method internationally. Measurement principle is that protein is a kind of nitrogenous organic compound, which can be decomposed into ammonia when heated together with sulfuric acid and the catalyst, and the ammonia is combined with sulfuric acid to generate ammonium sulphate. Then alkaline distillation is performed to make ammonia free, which is absorbed by boric acid and titrated with sulfuric acid or hydrochloric acid standard solution. According to the acid consumption multiplied by the conversion factor, the protein content can be calculated. Micro-Kjeldahl method is derived from titration analysis method of analytical chemistry [3].

At present, the classic Micro-Kjeldahl method is still applied as the statutory standard test method around the world, when testing the nitrogen content of the tested substance quantitatively. When the nitrogen content is determined by using the Micro-Kjeldahl method, manual operation is usually used. Manual determination of the protein content requires many intermediate steps and long operating time, which is prone to human error, resulting in a large deviation in measurement result; once an error occurs in the middle link, it may lead to the failure.

With the progress of electronic instruments and measurement science, the intelligent degree of electronic devices has been greatly improved. In the determination of the protein content of milk products, intelligent instrument is the developing direction in order to lift the measurement speed, improve accuracy and measuring instruments precision, and eliminate human factors; namely intelligent instruments are used to determine the nitrogen content.

The speeds of existing protein detection methods and instruments are too slow, not conducive to rapid detection of a large number of samples; in addition, instrument acquisition cost or testing cost is too high. The rapid detection equipment for dairy protein content is studied in this paper based on LPC2214 microcontroller and Micro-Kjeldahl method.

3. Working Principle of Micro-Kjeldahl Method

3.1. Overview of Titration Analysis

Titration analysis is a very important method in chemical quantitative analysis. Titration analysis generally refers to that the standard titration solution is added into the tested substance solution until the reaction is completed exactly, and then the content of the component in tested substance is calculated according to the concentration of the standard solution, the consumed volume, the measuring relationship between chemical reactions and the weight of the tested substances, *etc.* [4].

There are many titration analysis methods. When the tested substance cannot chemically react with the titrant directly, indirect ways of other reactions can be applied to measure the substance content. Micro-Kjeldahl method is to use indirect titration.

3.2. Principle of Micro-Kjeldahl Method

According to indirect titration analysis method, Micro-Kjeldahl method includes three steps:

(1) Digestion

In the determination of the nitrogen content in substance, the sample is generally treated properly, and various nitrogen compounds are decomposed into simple NH_4^+. A certain number of samples are selected, placed in the digestion tube, and digested sufficiently with concentrated sulfuric acid and catalysts (copper sulfate, potassium sulfate) at high temperature, to generate ammonium sulfate ($(NH_4)_2SO_4$).

Chemical reaction equation is:

$$2NH_2 - CH_4 + 2H_2SO_4 \rightarrow (NH_4)SO_4 + 2CO_2\uparrow + SO_2\uparrow + 3H_2O \quad (1)$$

(2) Distillation

Excessive concentrated *NaOH* solution is added to the completely decomposed samples after digestion containing NH_4^+ to be heated for distillation, to make NH_4^+ converted into NH_3.

Chemical reaction equation is:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O + 2NH_3\uparrow \quad (2)$$

Ammonia and water vapor released in the reaction are condensed by the condenser, collected in boric acid absorbing liquid in the receiving cup, and NH_3 distilled can be absorbed by excessive H_3BO_3 solution that needs no measurement to form distillation receiver solution.

Chemical reaction equation is:

$$NH_3 + H_3BO_3 \rightarrow NH_4H_2BO_3 \quad (3)$$

(3) Titration

HCL standard titration solution is used to titrate to generate $NH_4H_2BO_3$ solution, and the mixed solution of bromocresol green and methyl red is chosen as an indicator.

Chemical reaction equation is:

$$NH_4H_2BO_3 + HCL \rightarrow NH_4CL + H_3BO_3 \quad (4)$$

In the chemical reaction equation, the amount ratio of substances is equal to the ratio of stoichiometric numbers. According to the equation (4), at the stoichiometric point, the total number of moles of *HCL* that is completely reacted with $NH_4H_2BO_3$ is equal to the

number of moles of $NH_4H_2BO_3$, whereas, the weight of *N* in $NH_4H_2BO_3$ is the total weight of *N* contained in the tested substance. By chemical knowledge, the amount of *HCL* reacted with $NH_4H_2BO_3$ can be calculated *i.e*., the mole (mol) number, and the number of moles of $NH_4H_2BO_3$ can also be known, so that the content of nitrogen in the tested substance can be determined according to the number of moles of $NH_4H_2BO_3$. As for how to eliminate the end point errors in stoichiometric point and titration end, blank test method can be applied, which is to adopt distilled water for detection instead of the sample solution to be measured by analytical methods and measuring conditions, and then the same procedure for the test substance is conducted, and the blank value is subtracted from the consumption value of hydrochloric acid, so the volume number of *HCL* reacted with $NH_4H_2BO_3$ can be obtained. Then the content of nitrogen element in the tested substance can be determined in accordance with the relevant formulas [5].

Depending on the amount of chemical substances on the concentration, volume of solution, the consumed amount of *HCL* can be determined, and then the content of nitrogen element in the tested substance can be determined according to the amount of material and the molar mass.

Protein is a complex nitrogen-containing organic compound, mainly composed of various amino acids. The determination of the protein is also based on in Micro-Kjeldahl method currently. The protein content is measured through the determination of the nitrogen content in the material according to the fixed ratio of nitrogen in various proteins. The nitrogen content obtained by this method, includes other organic and inorganic nitrogen in addition to the nitrogen that does belong to protein constituents, so the protein content obtained by calculation is the content of crude protein. According to Micro- Kjeldahl method, the nitrogen content of the material can be measured. Then according to the nitrogen content multiplied by the corresponding conversion factor, the crude protein content can be obtained [6].

Based on the above analysis, the formula for the nitrogen content and the crude protein content in the tested substance can be drawn as follows.

Nitrogen content:

$$N(\%) = ((14.01 \times M \times (V - V_0)/1000)/W) \times K \times 100 = ((1.401 \times M \times (V - V_0))/W) \times K \quad (5)$$

Crude protein content:

$$P(\%) = ((14.01 \times M \times (V - V_0)/1000)/W) \times K \times 100 \times C = ((1.401 \times M \times (V - V_0))/W) \times K \times C \quad (6)$$

M: Amount of substance of *HCL* (mol) concentration (mol/l)
W: Weight of sample (g)
C: Conversion coefficient of crude protein
K: Correction factor for the instrument determination
V_0: Blank value (ml)
V: Titration volume of HCL (ml)

4. System Structure and Working Principle of Protein Rapid Detection Equipment

To design a system to automatically determine the nitrogen content of the material according to the Micro-Kjeldahl method is actually to realize four chemical reactions Kjeldahl method mentioned automatically and work out the final result automatically according to the formula derived in the introduction. Due to the special nature, digestion is not suitable to be conducted in the same instrument as distillation and titration; instead, digestion is conducted alone in another instrument. Rapid detection equipment of protein is actually to realize automatic control of two steps, distillation and titration.

4.1. Overall Structure and Process of Intelligent Azotometer

According to the latter three reaction formulas of Micro-Kjeldahl method, the overall structure diagram of distillation and titration is shown as Figure 1.

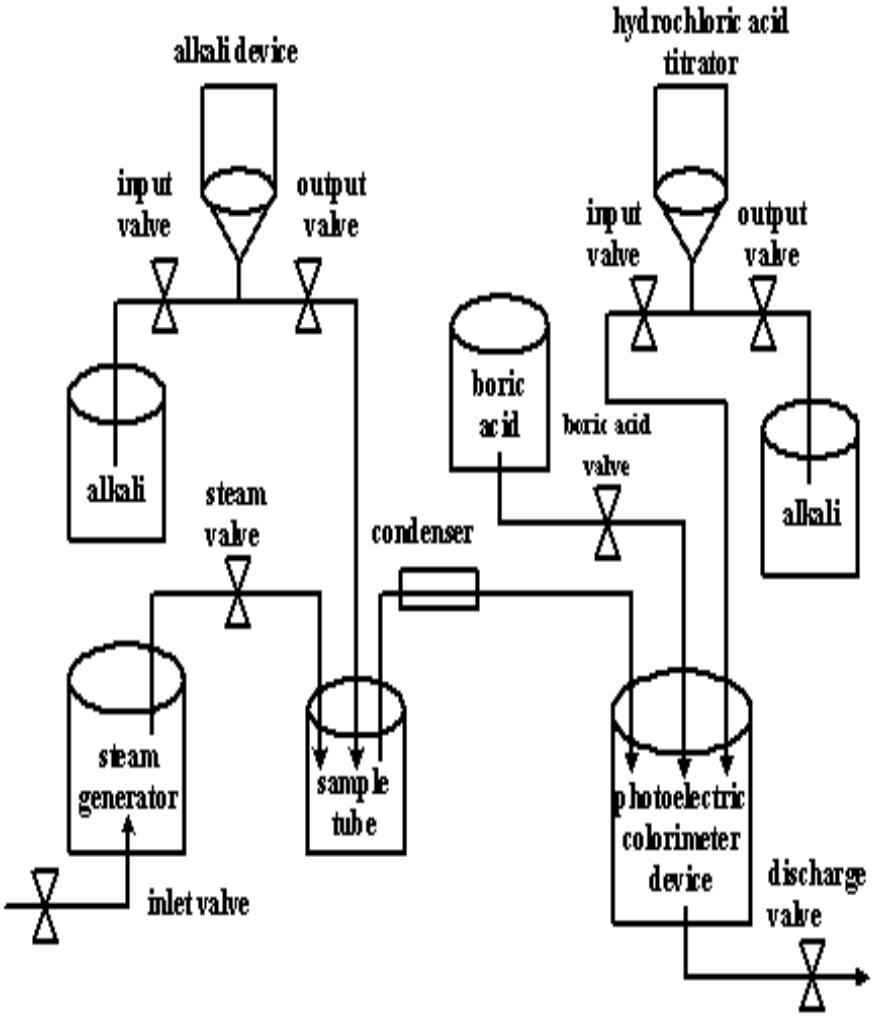

Figure 1. Overall Structure Diagram of Protein Rapid Detection Equipment

The whole system mainly consists of the embedded system based on ARM microcontroller LPC2214 from PHILIPS as control core, automatic steam generator, photoelectric colorimeter, automatic titrator, plus drainage system and so-on.

The embedded system with ARM microcontroller as control core receives and processes the signals from various sensors real-time and sends the control signal to take action. Meanwhile, it conducts the key processing of the keyboard, displays information and prints data depending on the situation at the scene. In addition, the crude protein content is calculated automatically by this system in the critical process—the automatic determination of the nitrogen content.

The required steam produced by steam generator in the distillation process, is similar to the micro-boiler, in which the water addition and heating processes are completed automatically under the control of *LPC2214*.

Photoelectric colorimeter is a large receiving cup, for containing distilled receiving liquid, and the neutralization titration reaction is also performed in the cuvette, where multiple level electrodes are installed to monitor the added amounts of the reactants, the

stirring device is used for mixing the solution well, and the use of photoelectric sensors is to determine the endpoint of the neutralization reaction.

Automatic titrator is the driving mechanism applied in the automatic titration of hydrochloric acid, of which the structure is similar to a syringe. The reciprocating movements of piston in titrator cavity form the inhalation and the extrusion actions of hydrochloric acid.

Plus drainage system is composed of several solenoid valves, to control the adding, moving and discharging of various liquids during the whole reaction.

Combined with the overall structure diagram and the principle of Micro-Kjeldahl method, the process of the entire system can be established as follows: the digested test tube containing the sample is put into the Azotometer and then the system is started. Firstly, boric valve is opened, the boric acid absorbing liquid containing indicator is added into photoelectric colorimeter vessel until the level reaches the amount of boric acid to close the valve after the control of electrode. Then, the output valve for alkali is opened, and the specified volume of *NaOH* solution is added to the sample tube by alkali device. Next, the steam generator begins to work automatically; the steam valve is opened, the entire distillation system is operated, a vigorous reaction occurs in alkaline solution and the sample under the action of steam, in which the generated ammonia gas enters the condenser with the steam, ammonia outflows from the condenser into the photoelectric colorimeter vessel, and absorbed by is boric acid absorption liquid; at this moment, due to the addition of ammonia, the liquid surface of photoelectric colorimeter vessel continues to rise, and when the liquid level reaches the control electrode of distillation receiving liquid, the distillation process is completed, the steam generator stops working, and the steam valve is closed. Finally, the titration process starts officially. The first step is to start the stirring motor and mix the solution uniformly; then the stepper motor is started to add hydrochloric acid into photoelectric colorimeter vessel, in which using a photoelectric device is used to test the signal of end point, and when the set value of titration end point is reached, the titration process stops, and the stepper motor is closed. The nitrogen content of the sample can be obtained by use of the titration steps forward of the stepper motor from the beginning to the end and related formulas [7].

4.2. Control Principle Analysis of Detection Equipment

So far, the nitrogen content has not been tested by use of sensitive components and measure conversion elements to forms a sensor like the detections of temperature and pressure. The determination of nitrogen content is actually to establish a set of automatic control system to test the nitrogen content in tested substance automatically according to the principle of Micro-Kjeldahl method.

The block diagram of the whole control system is shown in Figure 2.

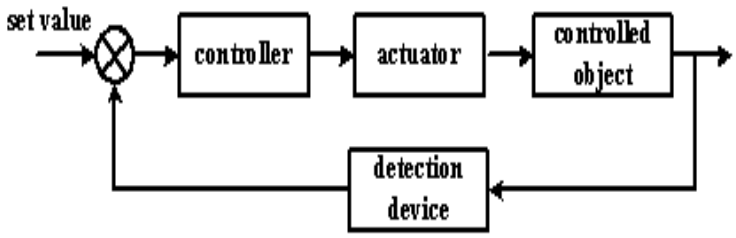

Figure 2. Block Diagram of the Control System

The whole control system is a closed loop control system, and the controller is ARM microcontroller, which is in charge of computing, processing and controlling; the actuator

is stepper motor, which drives automatic titrator to accurately titrate hydrochloric acid; the controlled object is automatic titrator, the controlled amount is the amount of standard hydrochloric acid added into colorimeter vessel and the detection device consists of spectral matching silicon photovoltaic cells and light-emitting diodes as well as signal conditioning circuit. Detection device detects the controlled volume indirectly, namely the volume of added hydrochloric acid. Detection device is to determine the titration endpoint by detecting the region of sudden color change in the solution. Set values must be set as in the sudden color change area in the solution.

According to the control requirements and the property of controlled volume, the control requirement of the detection system is the accurate determination of the titration endpoint signal, thus calculating hydrochloric acid titration volume by the number of steps traversed by a stepping motor. Controlled volume of hydrochloric acid is added into photoelectric colorimeter by a single direction. The difference value Δe between set and measured values of the system is decreasing in one direction, and when it reaches zero, the system reaches the titration endpoint.

5. Hardware Design of Protein Rapid Detector

According to the functional requirements of the equipment, the design of entire system should follow a design concept of top to down, big to small and coarse to fine, which is to divide the hardware into several modules according to the function levels of the system, namely, a modular design idea. In the hardware design, the entire system is divided into six major parts, host circuit module, input channel module, output channel module, human-machine interface component module, communication interface module and power management module.

The electrical principle block diagram of the control system is shown in Figure 3.

It is necessary to analyze the hardware requirements of the system in order to select the appropriate chip for the system among various types of embedded microcontrollers. In the hardware design, the convenience of the software design should be considered. After the analysis of hardware requirements, this system needs one analog input, 3 ways for controlling pulse signal supplied to the stepper motor, 30 switch outputs, 21 switch inputs, the micro-printer by use of bus control, two serial ports, I^2C interface, and reserved points (for system expansion). After analysis of various embedded microcontrollers in the market, the internal interrupt function, timer/ the number of counters, communication functions, memory capacity and other available internal functional modules need to be considered. After comprehensive consideration, finally a popular ARM microcontroller rich in resources is adopted. In addition, PHILIPS microcontroller is widely promoted in our country now by ZLGMCU Co., LTD. So ARM LPC2214 microcontroller from PHILIPS company is chosen as the control core of the whole system. The internal resources of the microcontroller can fully meet the requirement of the system in hardware.

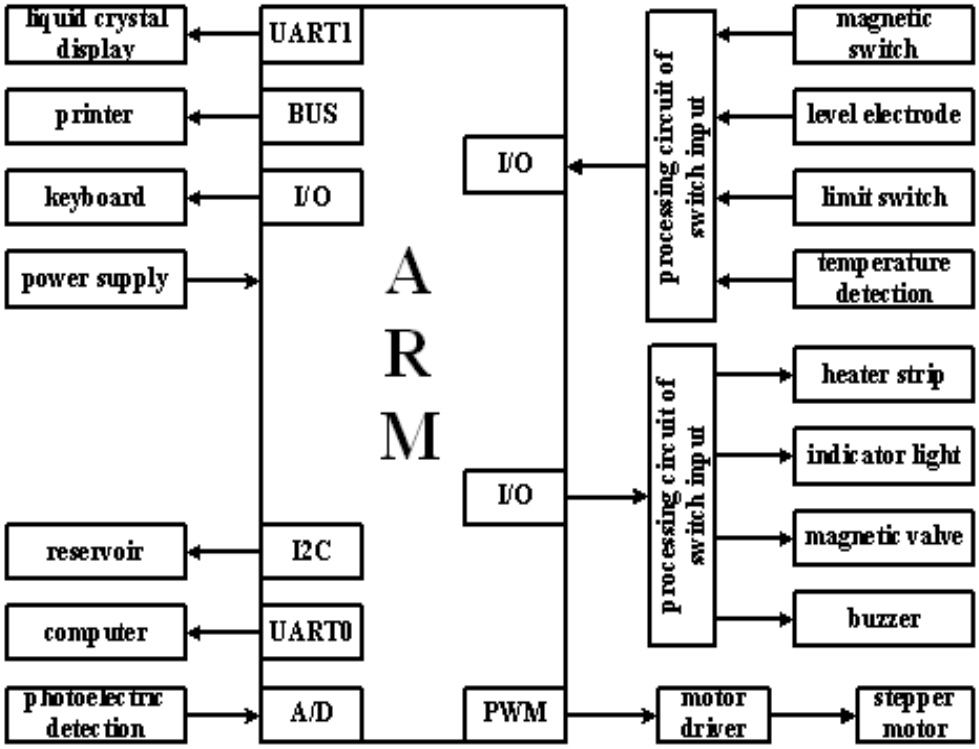

Figure 3. Electrical Principle Block Diagram of the Control System

6. Software Design for Rapid Detection of Protein

The system adopts proscenium and background system for software design, without transplanting operating system, and therefore, it comes to the design phase of the application after completing the design of startup code.

After the execution of start-up code, it jumps to the entrance of main ( ) function of the application program C language, that is, into the main program loop. During the execution of the main program loop, the appropriate subroutines are used in turn, waiting for the occurrence of corresponding interrupt to execute the interrupt service. The flow chart of the main program loop is shown in Figure 4.

Firstly, the system resources are initialized after entering the main loop program, and mainly internal functional modules of *ARM* are initialized. We must firstly initialize the pin connection module, and the multiplexed pins of *LPC2214* microcontroller are connected to the corresponding on-chip peripherals by setting registers *PINSEL0, PINSEL1* and *PINSEL2*. As for the requirement of pins set as the *GPIO* function, *IODIR* register is set, and each port can be set as output or input port. For output port, whether the initial state of the output port requires high or low levels should be considered, and high or low level at output port is set by *IOSET* or *IOCLR* register. The direction of the pin connected to the other functions is set automatically. Secondly, the *UART* serial port module must be initialized, I^2C bus module, timer module, *PWM* pulse width modulator, *A/D* analog-digital converter, vector interrupt controller and other internal modules. The initializations of these function modules are to set the appropriate registers in accordance with the requirements of the system.

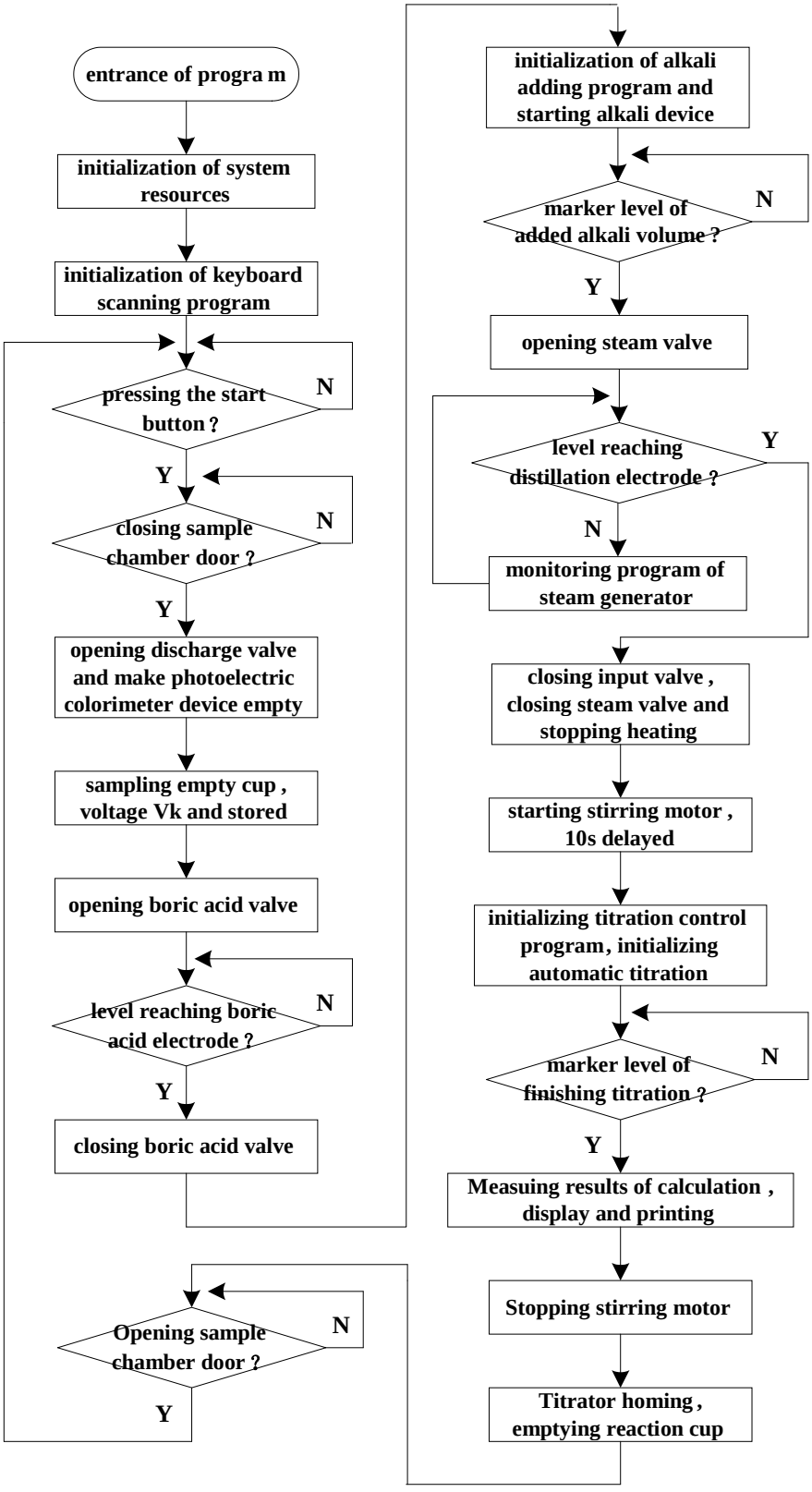

Figure 4. Flow Chart of the Main Program in System Loop

After the initialization of system is completed, the program enters a loop waiting for the start button is pressed. T1 interrupt program interrupts once by every 50ms to scan the keyboard, and in the scanning of the keyboard, when the corresponding button is pressed, the program will go to the corresponding key processing subroutine to perform the appropriate action. When the start button is pressed, the first step is to determine whether the sample compartment door is closed; only when the sample compartment door is closed, the program considers a sample tube has been placed, and then the work can be started. After the beginning of determination of the nitrogen content, the program firstly discharges the valve to make photoelectric colorimeter device empty. When the level of cup is below the minimum level of boric acid control electrode, 5s is delayed and then discharge valve is closed to ensure that the solution is completely empty in the cup. At this point, the program detects voltage signal Vk of light battery by second-level operational amplification, which is sampled for eight times and averaged value is stored in variables, which is the use of digital filtering technology. Due to the presence of interference, there is a shake in output voltage, so the arithmetic mean of the filtering technique is applied for smoothly processing the samples.

Then it is the process of specific implementation of the system. Firstly, absorption liquid of boric acid is added, boric acid valve is opened, to detect electrode signal of boric acid circularly, and the valve is closed when liquid level reaches boric acid electrode. Since there are fluctuations in the liquid level during the addition, the fluctuation-eliminating method with the same software delay as the keyboard detection is applied. The other switch detections in the system have adopted this method of software delay. Then the system starts alkali device, and then set signal is detected circularly by the program. In this process, interrupt signal is generated by the timer according to the system requirement, and in the interrupt service routine, a given I/O port is set and reset, to generate a pulse signal to control the stepping of stepper motor in driving alkali device. When the set signal of alkali volume is set, alkali-adding process comes to the end in the system. Next, the steam generator system is started, and distillation electrode signal is detected circularly by the program; if the liquid level of photoelectric colorimeter does not reach the distilled electrode, the program calls the steam generator to monitor the subroutine, to supply control steam. When the liquid level reaches the distillation electrode, the system will stop the supply of steam. Then the stirring motor is started, a time delay is taken so that the solution in the cup can be mixed well. The program continues to generate timer interrupt, to control the flip at corresponding I/O port to generating pulse supply for controlling stepper motor stirring device till the motor needs to be closed. And the system then starts automatic titrator to start the titration process; in the main loop, the program continues the loop detection of flag set signal of titration end, and when there is no set, PWM pulse width modulator of LPC2214 microcontroller is used to control PWM2 foot to output pulse signals in order to control the stepping of stepper motor, and meanwhile generating an interrupt signal. In addition, the titration control procedure is designed in interrupt service routine. When the flag set of titration is finished, stepper motor is stopped. And then calculation, display and print work are carried out. After the output, the program stops stirring motor, titration motor is homing, and the discharge valve is opened to make photoelectric colorimeter device empty. Finally, the user can start the next round of monitoring and control work after simply opening the sample chamber door and changing new samples.

7. Experimental Test

Different batches of whole milk powder and skimmed milk powder from one factory are taken as sample, in which 2 grams per sample is accurately weighed. Each batch of samples is tested by the detectors of different manufacturers and by the detector

developed in this paper respectively, and the obtained data are shown in Table 1 and Table 2.

Protein contents of different batches of whole milk powder:

Table 1. Protein Contents of different Batches of Whole Milk Powder

Different batches of whole milk powder	1	2	3	Mean value of true protein
1	25.34%	25.83%	25.90%	25.69 ± 0.31%
2	25.65%	25.52%	25.87%	25.68 ± 0.18%
3	25.67%	25.75%	25.73%	25.72 ± 0.04%
4	25.16%	25.12%	24.64%	24.97 ± 0.29%
5	24.37%	24.63%	23.90%	24.30 ± 0.37%

Protein contents of different batches of skimmed milk powder.

Table 2 Protein contents of different batches of skimmed milk powder:

Different batches of skimmed milk powder	1	2	3	Mean value of true protein
1	35.44%	35.19%	35.24%	35.29 ± 0.13%
2	35.72%	35.57%	36.29%	35.86 ± 0.38%
3	35.86%	35.71%	35.98%	35.85 ± 0.14%
4	33.36%	33.29%	33.35%	33.33 ± 0.04%
5	34.05%	33.98%	34.06%	34.03 ± 0.04%

As can be seen from the experimental data, protein contents of different batches of milk powder tested by various instruments are substantially the same, which suggests the detector designed in this paper has high accuracy, and small error, which meet the system requirement in design.

8. Conclusions

In this paper, the LPC2214 microcontroller with ARM structure is taken as the control core, and a new type of intelligent instrument for determining protein content in dairy products is designed by analyzing Micro-Kjeldahl method and the control principle of system. This system has the functions of adding certain volume of lye, adding certain volume of boric acid absorption liquid, automatically generating steam required, automatically titrating hydrochloric acid solution, accurately determining the endpoint and conveniently setting parameters, which realizes the digital and intelligent design in determining the protein content in dairy products. Experimental results show that the system is stable, with broad application prospects.

Acknowledgments

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