JOURNAL OF
EMERGING INVESTIGATORS

Improving measurement of reducing sugar content in carbonated beverages using Fehling's reagent

Yun Zhang[1], **Qiming Chen**[2]
[1]Shanghai Foreign Language School Affiliated to Shanghai International Studies University Shanghai, China
[2]School of Chemistry and Molecular Engineering, East China Normal University Shanghai, China

SUMMARY

Growing dietary sugar intake has been associated with the increasing global prevalence of obesity and diabetes. As a result, several health organizations recommend reduction in daily sugar. In the sugar industry, the Fehling's method is still the most commonly used for determining the reducing sugars in sugar products. However, it is difficult to control the experimental conditions and determine the terminal point of titration by color change. Therefore, this investigation explored the optimal concentrations of the constituents and reaction temperature of Fehling's reaction and determined the reducing sugar content of three best-selling beverages in China using the optimal method. The results showed that the optimal concentrations of Fehling's reagent are: solution A: 0.075 mol/L copper sulfate; solution B: 0.075 mol/L potassium sodium tartrate, and 1.5 mol/L sodium hydroxide. The optimal temperature for the reaction is 75 °C. The content of reducing sugar in the three beverages, Maidong, Xiaoming tongxue, and Coco milk tea determined by the improved Fehling's reagent is 1.801 g/100 ml, 5.483 g/100 ml, and 9.956 g/100 ml, respectively. The improvement of Fehling's reagent might enhance the accuracy and efficiency for reducing sugar test. The high content of reducing sugar in these best-selling beverages may allow consumers to better understand the nutritional content of their favorite drinks and may help them decrease their sugar intake.

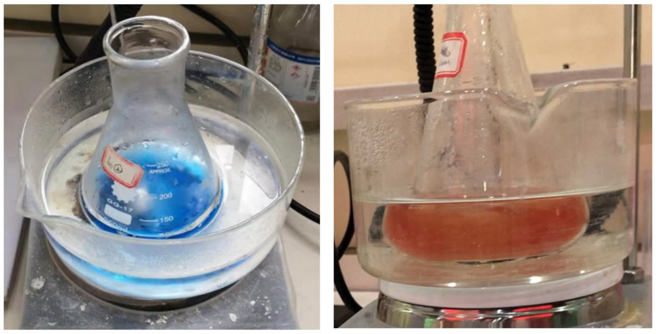
INTRODUCTION

In recent years, intake of high sugar desserts and beverages has been increasing dramatically, leading to high blood sugar and associated morbidities, such as caries, obesity, and diabetes (1). The recommendation from World Health Organization (WHO) is supported by evidence showing higher rates of dental caries when the intake of free sugars is above 10% of total energy intake compared with an intake of free sugars below 10% of total energy intake (2). In nutrition research, the different carbohydrates contained in a diet is often correlated with their specific metabolic behavior (3). For example, glucose, fructose, sucrose are important in biology. The living cell uses them as source of energy and metabolic intermediates. Separation methods have allowed for greater accuracy of sugar analysis in foods. Individual sugar measurements can be summed to calculate the total sugar content for the nutrition information panel. Gas chromatography (GC) is a popular method for carbohydrate analysis and is very sensitive and accurate. High performance liquid chromatography (HPLC) is another established analytical method for measuring individual sugars in many foods. However, each method has its disadvantages including coelution, tedious sample preparation, and intolerance to salt or acid leading to short column life and high running costs (3-8). Sugar in food includes both reducing and non-reducing sugars. Reducing sugars mainly include glucose, fructose, maltose, and lactose. Reducing sugar refers to a monosaccharide or disaccharide with a free aldehyde group, ketone group, or hemiacetal hydroxyl. Its reductive effect is due to the enolization (the conversion of a ketone into an enol) of sugars. Non-reducing sugar generally refers to sucrose. Sucrose can be hydrolyzed to equal amounts of glucose and fructose. Most foods contain a mixture of sugars rather than a single type of sugar.

Fehling's solution is used as a chemical test to differentiate between water-soluble aldehyde and ketone functional groups, and as a test for monosaccharides. It can be used to screen for glucose in urine, thus detecting diabetes. It is still most commonly used for determining reducing sugars in sugar products. The test was developed by German chemist Hermann von Fehling in 1849 (9). Fehling's reagent can be used to measure the amount of reducing sugars and calculate the glucose equivalent of the starch sugar. It was reported that the classical Fehling's concentrations are 6.928 g/100 ml of copper sulfate, 34.6 g/100 ml of potassium sodium tartrate, and 10 g/100 ml of sodium hydroxide (10).

Despite its widespread use, Fehling's method is glucose specific (e.g. glucose-oxidase peroxidase) and unable to distinguish between individual reducing sugars (11). In reducing sugar assays, the quantity of product formed and measured is not equivalent to sugar content, and different sugars yield different color intensities. This inaccuracy and ambiguity shows that the method is considerably more complicated than it appears and difficult to interpret (4).

Many novel methods have been proposed to measure reducing sugar content. However, in many routine analytical laboratories, particularly in the sugar industry, Fehling's method is still the most commonly used protocol for determining reducing sugars in sugar products (12). Manufacturers and researchers are commonly concerned about determining the reducing sugar content quickly and accurately. As a classical method of reducing sugar content determination, Fehling's method is widely used because it is inexpensive, quick, and technically easy to perform. In order



Fehling's solution before reducing

Fehling's solution after reducing(End poind)

Figure 1. The color change of Fehling's solution (before and after reducing). Before reduction, the solution was sky blue. After reduction, the solution was brick red.

to increase the accuracy, stability, and convenience of Fehling's method, many of the modifications determined the most suitable temperature, the influence of different concentrations, and the ideal indicator for reaction completion (13-16). However, strict control of the experimental conditions, such as the rate of heating or the alkalinity and strength of the reagent, in a non-automated setting is necessary to obtain repeatable and reproducible results (4, 17). Furthermore, it is difficult to determine the terminal point of titration by color change, which may cause variation in the quantitative analysis results of reducing sugar. Therefore, it is important to study the ideal indicator for end point, the optimal formula and reaction temperature of the Fehling's method to improve the effect and accuracy. This research aimed to use the color change of the Fehling's reagent to indicate the end point instead of adding methylene blue which is a commonly used indicator for oxidation-reduction reactions, and to explore the optimal concentrations and reaction temperature. Then we used these optimal test conditions to determine the reducing sugar content of three best-selling beverages in China. We hypothesized that ideal concentrations and reaction temperature would result in better accuracy and efficiency in reducing sugar tests and that the high content of reducing sugars in best-selling beverages is a serious concern for public health.

RESULTS

The determination of reducing sugars by Fehling's method is based on the reducing action of sugars on alkaline copper (II) solution. Changing only one variable at a time to see what the optimal concentrations and temperatures are for the Fehling's reagent. The concentration and temperature corresponding to the lowest reaction time and the brick red color are chosen as optimum (**Figure 1**). Each experiment is repeated three times, and its mean±standard deviation (SD) is recorded as experimental results.

Groups	Copper sulfate（mol/L）	Potassium sodium tartrate tetrahydrate (mol/L)	Sodium hydroxide (mol/L)	Color of terminal point	Titration terminal time (Minute)
1	0.05	0.1	0.2	Brick Red	3.92±0.032
2	0.075	0.1	0.2	Brick Red	1.92±0.017
3	0.1	0.1	0.2	Brick Red	7.35±0.051
4	0.125	0.1	0.2	Brick Red	15.55±0.082
5	0.15	0.1	0.2	Color unchanged	—

Table 1. Results of standard glucose solution on the determination of reducing sugar with varying copper sulfate. Under the condition of 0.1 mol/L potassium sodium tartrate tetrahydrate, 0.2 mol/L sodium hydroxide, when concentration of copper sulfate was 0.075 mol/L, the titration terminal time was the quickest (1.92±0.017 minute).

Groups	Sodium Hydroxide（mol/L）	Copper sulfate (mol/L)	Potassium sodium tartrate tetrahydrate (mol/L)	Color of terminal point	Titration terminal time (Minute)
1	0.1	0.075	0.1	Sky blue	No titration terminal
2	0.4	0.075	0.1	Brick Red	1.67±0.052
3	0.6	0.075	0.1	Brick Red	1.42±0.034
4	0.8	0.075	0.1	Brick Red	1.28±0.045
5	1.5	0.075	0.1	Brick Red	1.08±0.014
6	2.0	0.075	0.1	Brick Red	1.32±0.019
7	2.5	0.075	0.1	Brick Red	1.28±0.017

Table 2. Results of standard glucose solution on the determination of reducing sugar with varying sodium hydroxide. Under the condition of 0.075 mol/L copper sulfate, 0.1 mol/L potassium sodium tartrate tetrahydrate, when concentration of sodium hydroxide was 1.5 mol/L, the titration terminal time was the quickest (1.08±0.014 minute).

To determine the effect of varying the amounts of copper sulfate in Fehling's solution, we added Fehling's solution containing various concentrations of copper sulfate while keeping concentrations of potassium sodium tartrate tetrahydrate, sodium hydroxide constant. When the concentration of copper sulfate was 0.075 mol/L, the reaction between Fehling's reagent and glucose showed the most obvious brick red color and the reaction time was the shortest (**Table 1**). Therefore, we determined that 0.075 mol/L copper sulfate is the optimal concentration to use in Fehling's reagent.

The concentration of sodium hydroxide influences the rate and amount of oxidation of the sugars. Based on the discoloration time and precipitation time, when the concentration of sodium hydroxide was 1.5 mol/L, the reaction was the fastest and the color change was the most obvious (**Table 2**). Therefore, 1.5 mol/L sodium hydroxide is considered as the optimal concentration. Up to a certain point, increasing concentration of hydroxyl ions increases oxidation. From that point onwards, a further increase of hydroxyl ions tends to decrease oxidation. The most likely explanation for this phenomenon is that the decrease in reduction is due to the formation in large quantities of compounds of lower reducing power.

To investigate the optimal concentration of potassium sodium tartrate, we added various amounts of potassium

Groups	Potassium sodium Tartrate（mol/L）	Copper sulfate（mol/L）	Sodium hydroxide（mol/L）	Color of terminal point	Titration terminal time (Minute)
1	0.05	0.075	1.5	Brick Red	1.42±0.017
2	0.075	0.075	1.5	Brick Red	0.97±0.009
3	0.15	0.075	1.5	Brick Red	1.17±0.007
4	0.2	0.075	1.5	Brick Red	1.00±0.014

Table 3. Results of standard glucose solution on the determination of reducing sugar with varying potassium sodium tartrate. Under the condition of 0.075 mol/L copper sulfate, 1.5 mol/L sodium hydroxide, when concentration of potassium sodium tartrate tetrahydrate was 0.075 mol/L, the titration terminal time was the quickest (0.97±0.009 minute).

sodium tartrate to constant amounts of alkali and copper sulfate. According to the darkening time, complete discoloration time, and precipitation time in the table, the quickest reaction group is 0.075 mol/L potassium sodium tartrate (**Table 3**). With this concentration of reagent, the brick red precipitate is the most apparent by eye. Therefore, when the concentration of potassium sodium tartrate in solution B is 0.075 mol/L, the effect of Fehling's reagent is optimal.

Groups	Temperature（℃）	Copper sulfate（mol/L）	Potassium sodium tartrate Tetrahydrate（mol/L）	Sodium hydroxide（mol/L）	Color of terminal point	Titration terminal time (Minute)
1	55	0.075	0.075	1.5	Brick Red	1.42±0.008
2	65	0.075	0.075	1.5	Brick Red	1.33±0.018
3	75	0.075	0.075	1.5	Brick Red	0.33±0.004
4	80	0.075	0.075	1.5	Brick Red	1.17±0.011
5	85	0.075	0.075	1.5	Brick Red	1.33±0.014
6	95	0.075	0.075	1.5	Brick Red	1.33±0.009

Table 4. Results of standard glucose solution on the determination of reducing sugar with varying reaction temperature. Under the condition of 0.075 mol/L copper sulfate, 1.5 mol/L sodium hydroxide, 0.075 mol/L potassium sodium tartrate tetrahydrate, when reaction temperature was 75 °C, the titration terminal time was the quickest (0.33±0.004 minute).

Then, we used the optimal concentrations to explore the optimal temperature and heating time. The temperature and time of heating exert a great influence on the oxidation of reducing sugar. At 75 °C, the reaction effect is the fastest and the color change is the most obvious (**Table 4**). Therefore, 75 °C reaction temperature is considered the optimum.

Next, we used the optimal concentrations of Fehling's reagent and temperature to determine the content of reducing sugar in standard glucose solution and the three beverages — Maidong (a kind of sports drink similar to gatorade), Xiaoming tongxue (sweetened tea drink), and Coco milk tea (made-on-site beverage) (**Figure 2**) —to be 1.765 g/100 ml, 1.801 g/100 ml, 5.483 g/100 ml, and 9.956 g/100 ml, respectively (**Table 5**). There is a discrepancy between the reducing sugar concentration of Maidong that we measured (1.801 g/100 ml) and the sugar concentration labelled on the tag (4.9 g/100 ml). This discrepancy also occurs with Xiaoming tongxue since the reducing sugar concentration that we tested 5.483 g/100 ml is different from 10.1 g/100 mL labelled on the tag. This is due to the fact that we only detected the content of reducing sugar, though other kinds of sugar such as non-reducing sugar are also present in the beverages. For this reason, the reducing sugar concentration is less than the sugar concentration labelled on the tag. Since Coco milk tea is a made-on-site beverage, there is no sugar label on the bottle.

Groups	Sugar content on tag（g/100mL）	Reducing sugar content by Fehling's reagent（g/100mL）
Standard Glucose Solution	1.802	1.765±0.021
Maidong	4.9	1.801±0.015
Xioming Tongxue	10.1	5.483±0.018
Coco Milk Tea	Not available	9.956±0.019

Table 5. Reducing sugar content in three beverages determined by improved Fehling's reagent. Among three beverages, Coco milk tea (made-on-site beverage) showed the highest reducing sugar content, up to 9.956±0.019 g/100mL.


Mai dong
Xiaoming Tongxue
Coco milk tea

Figure 2. Three best-selling beverages. Maidong (a kind of sports drink similar to gatorade), Xiaoming tongxue (sweetened tea drink), and Coco milk tea (made-on-site beverage).

DISCUSSION

This study aimed to optimize Fehling's solution reaction conditions in order to overcome limitations and accurately determine the titration end point. Our experiment supported our hypothesis. We found that the optimal conditions were 75 °C, 0.075 mol/L copper sulfate solution, 0.075 mol/L potassium sodium tartrate, and 1.5 mol/L sodium hydroxide. Furthermore, we found that instead of adding methylene blue, color change of the Fehling's reagent could accurately indicate endpoint.

Sparing reagents is a great concern in the experiment. The original Fehling's method required large amounts of reagents. This resulted in large amount of associated metal waste products (13, 18). Compared with the classical formula, our method reduced the required amounts significantly, for example, copper sulfate (1.197 g/100 ml vs. 6.928 g/100 ml), potassium sodium tartrate (2.117 g/100 ml vs 34.6 g/100 ml), and sodium hydroxide (5.994 g/100 ml vs 10 g/100 ml) (10).

We optimized the copper sulfate concentration first and then used that concentration for the other tests. We chose the concentrations of sodium hydroxide (0.1 mol/L) and potassium sodium tartrate tetrahydrate (0.2 mol/L) for optimizing the copper sulfate concentration based on the literature (13). It was reported that a ratio of 5–6 of sodium hydroxide to 1 of copper by weight was required (13). We observed that the optimal concentrations in the current study give a 1:1:2 molar ratio of reagents among copper sulfate, potassium sodium tartrate tetrahydrate, and sodium hydroxide. Further, the ratio of 1 molecule of glucose to 5 of copper was regarded constant by Fehling for complete reaction and was so employed. However, the ratio was not constant, but varied according to the amount of copper in solution (14). Due to the high concentration of copper (I) ions in the classical Fehling's reagent, the reaction was not complete and the experimental color change phenomenon was not obvious to observe (10). Under the current modified experimental conditions, the precipitate was brick red and the color change was more obvious to judge.

Temperature exerts a great influence on the oxidation of dextrose. The chemical reaction between glucose and Fehling's reagent solution is an oxidation-reduction reaction which is slow at room temperature. High temperature can speed up this reaction (4). Temperature increases reduction rate, and it appears complete below 90 °C (13). In the current study, the optimal temperature was 75 °C. Though temperatures lower than 75 °C may have led to complete reduction, the heating time required to give a reasonable amount of copper for quantitative determination would be excessive. On the other hand, at higher temperatures, the difficulty of accurately maintaining the temperature of the bath interferes with the accuracy of short digestions.

To prevent harm to human health caused by excessive intake of sugar, many countries and regions have taken measures such as requiring labelling of sugar content on the nutrition labels of food and drinks and reminding consumers to decrease their sugar intake (19-21). However, labelling of sugar in food is still not required by many countries, including China. Furthermore, there is no labelling of sugar for made-on-site beverages which are very popular among teenagers. This is a global issue. The current study shows that the content of reducing sugar in the best-selling beverages is very high, especially in the made-on-site beverages (Coco milk tea 9.956 g/100 ml compared to Maidong 1.801 g/100 ml, Xiaoming tongxue 5.483 g/100 ml). In addition to reducing sugar content, it is also important to determine the level of non-reducing sugar content of foods. Non-reducing sugar generally refers to sucrose that might be present in common beverages. More advanced methods for separation of different reducing sugars, for example gas chromatography, high performance liquid chromatography have allowed for greater accuracy of individual sugar measurements in foods. Raising the awareness of teenagers to reduce sugar intake, and appeal to label the sugar content and rank of free, low, reduced, light/lite, or high on the tags of made-on-site beverage is very necessary in order to prevent and control obesity and diabetes.

The main limitation of the present study is an inaccurate quantitative endpoint measurement. The errors involved are lack of exact terminal point and personnel errors. It is required that the determination process of standard solutions and samples should be calibrated. A more accurate measurement could be obtained with a spectrophotometer. It would be interesting to run a control experiment using the standard Fehling's reagent conditions used in industry and comparing that to the optimized Fehling's constant. In future experiments, with more resources at hand, we would add the tests above to further substantiate our experimental results.

We believe that the improved Fehling’s measurement of reducing sugar content in carbonated beverages with quick and obvious terminal point, better accuracy and efficiency provides a great contribution to the available literature.

MATERIALS AND METHODS

Fehling's solution is comprised of two separate solutions, known as Fehling's A and Fehling's B. Fehling's A is a blue aqueous solution of copper sulfate pentahydrate crystals, while Fehling's B is a clear solution of aqueous potassium sodium tartrate (also known as Rochelle salt) and a strong alkali (sodium hydroxide). Fehling's solution is always prepared fresh in the laboratory. The main reagents of Fehling’s solution include copper sulfate pentahydrate solid, potassium sodium tartrate tetrahydrate solid, sodium hydroxide (flake) solid, and anhydrous glucose solid.

In each experiment, 10 mL Fehling’s solution A and 20mL Fehling’s solution B were prepared and were mixed in a conical flask by being stirred slowly and constantly. Then 25mL of 0.1 mol/L glucose solution was added into the conical flask. This was heated continuously in a water bath until the solution turned brick red.

The indicator for reaction completion

The classical Fehling’s method for the determination of reducing sugars is based on the colorimetric measurement of the oxide or the free metal formed. This method relies on reduction of copper (II) tartrate in a strongly alkaline medium using methylene blue as an indicator. The titration end point is when methylene blue turns to colorless. It is very likely to be affected by factors such as titration speed, which may cause the outcome bias. The method requires visual end point detection, which is difficult to observe, especially in dark-colored solutions of raw sugars and other sugar products, such as molasses (12). Instead of adding methylene blue, color change of the Fehling’s reagent can be used to indicate endpoint. We followed the procedure by Zang *et al.* (16). In our detection procedure, solution A includes copper sulfate solution and solution B includes sodium hydroxide, potassium sodium tartrate. After mixing solution A and solution B, a light blue copper hydroxide $Cu(OH)_2$ precipitate is formed. Then this precipitate reacts immediately with potassium sodium tartrate, forming a dark blue potassium sodium tartrate copper complex. When the Fehling's solution and reducing sugar are

Sample	Standard glucose Solution (1.802 g/100mL)	Standard glucose Solution (3.604 g/100mL)	Standard glucose Solution (5.406 g/100mL)
1	1.625	3.609	5.403
2	1.781	3.612	5.401
3	1.822	3.702	5.358
4	1.917	3.583	5.402
5	2.011	3.602	5.391
6	1.762	3.605	5.405
Mean	1.820	3.619	5.393
SD	0.054	0.042	0.018
AE (g/100mL)	0.017	0.014	0.013

Table 6. Different concentrations of standard glucose solution calibrated using the improved Fehling's method. SD = standard deviation, AE = absolute error. The absolute error of calibration standard glucose solution 1.802, 3.604, and 5.406 g/100mL were 0.017, 0.014, 0.013 g/100mL respectively.

heated, the potassium sodium tartrate copper is reduced to a brick red precipitate of cuprous oxide (Cu_2O). The bistartratocuprate (II) complex oxidizes the aldehyde to a carboxylateanion, and the copper (II) ions of the complex are reduced to copper (I) ions. Red copper (I) oxide then precipitates out of the reaction mixture, which indicates a positive result. The reducing sugar is oxidized. Change in blue color of the solution to brick red Cu_2O indicates that the end point is reached. The chemical reaction equations (10) are as below:

$$CuSO_4 + 2NaOH = Cu(OH)_2 \downarrow \text{(light blue)} + Na_2SO_4$$

$$CH_2OH(CHOH)_4CHO + 2Cu(OH)_2 = CH_2OH(CHOH)_4COOH + Cu_2O\downarrow \text{(brick red)} + H_2O$$

The calibration for the improved Fehling's method

The calibration for the improved Fehling's method of the current study was implemented using different known concentrations of standard glucose solution to ensure the accuracy of improved Fehling's formula (0.075 mol/L copper sulfate solution, 0.075 mol/L potassium sodium tartrate, and 1.5 mol/L sodium hydroxide). Absolute error less than 0.05 g/100 mL is considered accurate (22). The results of absolute error of different known concentrations of standard glucose solution tested by the current improved Fehling's method are 0.017 g/100 mL, 0.014 g/100 mL, and 0.013 g/100 mL, respectively (**Table 6**). The results show that the improved method is more accurate than the classical Fehling's method.

Detection of reducing sugar content in beverage by the improvement Fehling's reagent

Using the optimal Fehling's reagent concentrations explored above, we determined the reducing sugar content in the three best-selling beverages in China: Maidong, Xiaoming tongxue, and Coco milk tea. We took 25 mL sample from three beverages respectively, and added water to dilute it to 250 mL, 10% solution. We then recorded the volume of beverages and the volume of 0.1 mol/L glucose solution consumed in order to determine the reducing sugar content as follows (23):

$$\text{Reducing sugar content (mg/mL)} = \frac{(\mathrm{A} - \mathrm{B})\ \text{mL} \times \text{concentration of glucose solution (mg/mL)}}{\text{Volume of titrated beverage solution(mL)}} x\ 10$$

Received: January 17, 2020
Accepted: May 20, 2020
Published: July 21, 2020

REFERENCES

1. Zhang Y, Zhou W, Li B. Determination of Sugar Alcohols Sweeteners in Sugar-free Food by Derivatization Capillary Gas Chromatography. *Chinese Journal of Analytical Chemistry.* 2013, 41(6): 911-916.

2. World Health Organization. Guideline: Sugars intake for adults and children. 2015. *World Health Organization.* https://www.who.int/nutrition/publications/guidelines/sugars_intake/en/17 Codex Alimentarius Commission. CAC/GL 2-1985 Guidelines on Nutrition Labelling 2015

3. Southgate, D. A. T., Introduction. In Determination of Food Carbohydrates, 2nd ed.; Southgate, D. A. T., Eds; *Elsevier Applied Science*. London, UK, 1991, pp 1-7.

4. MR Toutounji, MP Van Leeuwen, JD Oliver *et al.* Quantification of sugars in breakfast cereals using capillary electrophoresis. *Carbohydrate Research*. 2015, 408: 134-141.

5. Schenk J., Nagy G., Pohl N., *et al*. Identification and deconvolution of carbohydrates with gas chromatography-vacuum ultraviolet spectroscopy. J*ournal of Chromatography A*. 2017, 1513: 210-221.

6. Hetrick E.M., Kramer T.T., Risley D.S. Evaluation of a hydrophilic interaction liquid chromatography design space for sugars and sugar alcohols. *Journal of Chromatography A*. 2017, 1489: 65-74.

7. Lindqvist D.N., Pedersen H., Rasmussen L.H. A novel technique for determination of the fructose, glucose and sucrose distribution in nectar from orchids by HPLC-ELSD. *Journal of Chromatography. B*. 2018, 1081-1082: 126-130.

8. Ma C., Sun Z., Chen C., *et al*. Simultaneous separation and determination of fructose, sorbitol, glucose and sucrose in fruits by HPLC-ELSD. *Food Chemistry.* 2014, 145: 784-788.

9. H. Fehling, The quantitative determination of sugar and starch by means of copper sulfate. *Annalen der Chemie und Pharmacie.* 1849, 72 (1): 106-113.

10. Gao HS, Song XQ, Wang GZ. Improving method of direct titration on Fehling'sreagent to determining content of sugar in fruit and vegetable. *Journal of Hebei Agrotechnical Teachers College.* 1987, 1(4): 63-66. In Chinese.

11. FitzGerald, J, Vermerris, W. The utility of blood glucose meters in biotechnological applications. *Biotechnology and Applied Biochemistry.* 2005, 41(3): 233-239.

12 Alexander PW Hartati RD Curtin J. Automated potentiometric end point determination in the Lane-Eynon titration of reducing sugars. *Electroanalysis*. 1989, 1 (3):263-269.

13. Quisumbing, FA., Thomas, AW. Conditions affecting the

quantitative determination of reducing sugars by fehling solution. *J. Am. Chem. Soc.* 1921, 1503-1526.

14. Zhang WJ. Biochemical research technology of glycoconjugates. [M] *Zhejiang University Press.* 1994: 7-8. In Chinese.

15. Li XM, Yang JH, Zhang LQ. Comparisons of Determination Approaches of Reducing Sugar. *Shandong Science.* 2008, 21(2):18. In Chinese.

16. Zang JK, Li BC, Zhang ZX. National standard 5009.7-85 method for determination of reducing sugar in foods is improved. *Acat scientiarum naturalium universitatis Chengduensis.* 1997, 3:25-28. In Chinese.

17. Southgate, DAT. In Determination of Food Carbohydrates. *Elsevier Applied Science*. London, UK, 1991, pp 9-33.

18. Liu M, Li CJ. Catalytic Fehling's Reaction: An Efficient Aerobic Oxidation of Aldehyde Catalyzed by Copper in Water. *Angew Chem Int Ed Engl*. 2016, 26;55(36):10806-10810.

19. Codex Alimentarius Commission. *FAO & WHO*. CAC/GL 2-1985 Guidelines on Nutrition Labelling 2015.

20. Brummer, Y.; Cui, S. Understanding Carbohydrate Analysis. In Food Carbohydrates, 1st. *CRC Press*. Florida, USA, 2005; pp 74-89.

21. Food Standards Australia New Zealand. *Standard 1. 2. 7 Nutrition, health and related claims*. 2013.

22. Yang LE, Peng XG, Yang QW, *et al*. Improvement of the determination of reducing sugar with Fehling's reagent method. *China Brewing*. 2010; 5(218):160-161. In Chinese.

23. Chen JB, Ji Yi, Zhao SJ, et al. Experience on the determination of soluble sugar in fruits by Fehling’s reagent. *Tropical Agricultural Science & Technology*. 1994, 19-22. In Chinese.