HiLL PUBLISHING

International Journal of Food Science and Agriculture, 2024, 8(3), 127-130
https://www. hillpublisher. com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Comparison of Detection Methods for Lactoferrin Iron Saturation

Yanjun Xia*, Wei Tan, Li Lan, Genna Ba

Inner Mongolia Yili Industrial Group Co., Ltd., Hohhot 010080, Inner Mongolia, China.

How to cite this paper: Yanjun Xia, Wei Tan, Li Lan, Genna Ba. (2024). Comparison of Detection Methods for Lactoferrin Iron Saturation. *International Journal of Food Science and Agriculture*, 8(3), 127-130.
DOI: 10.26855/ijfsa.2024.09.006

Received: July 18, 2024
Accepted: August 15, 2024
Published: September 13, 2024

Corresponding author: Yanjun Xia, Inner Mongolia Yili Industrial Group Co., Ltd., Hohhot 010080, Inner Mongolia, China.

Abstract

Due to the extensive application of lactoferrin in food, medicine, and other fields, quality control is particularly important. As a key quality indicator of lactoferrin, the accurate detection of iron saturation is crucial for ensuring the quality and efficacy of lactoferrin products. However, there is currently no standardized method for detecting iron saturation in lactoferrin within the industry, leading to variations in test results as different manufacturers may employ different detection techniques. Therefore, selecting more accurate and reliable methods for detecting iron saturation in lactoferrin is essential for quality control and the standardized production of lactoferrin products. This paper aims to provide a scientific reference for researchers in choosing the appropriate detection method for lactoferrin iron saturation. The study examines the current widely used detection methods for lactoferrin iron saturation, including saturation analysis (Method 1) and ultraviolet-visible spectrophotometry (Method 2). Through in-depth analysis and comparison of the principles, accuracy, and operational complexity of these methods, the results indicate that Method 1 generally yields higher iron saturation values and demonstrates better stability and consistency in repeated measurements. This comparative study aims to offer valuable references for the precise detection of lactoferrin iron saturation, thereby promoting research and development in related fields.

Keywords

Lactoferrin iron saturation; Detection method; Comparison

Introduction

Lactoferrin (Lf), as a non-heme iron-binding glycoprotein widely found in mammalian milk, not only has a powerful immune regulation function but also shows an important physiological role in iron metabolism, antibacterial, antioxidant, and other aspects [1]. Among them, the iron saturation of lactoferrin (namely the ability of lactoferrin to bind iron) is one of the important indicators to measure its biological activity and function. Lf has a higher ability to bind iron and iron saturation is usually measured by A465nm/A280nm. However, it has been shown that it is easy to introduce large errors using this method due to the large difference in the absorbance values of the two wavelengths used [2]. Therefore, accurate and efficient detection of the iron saturation of lactoferrin is important for understanding its mechanism of action in organisms and the development of relevant functional foods and drugs [3].

With the continuous development of science and technology, the detection methods of lactoferrin iron saturation are increasingly diversified and precise [4]. From the traditional chemical analysis method to the modern instrument analysis technology, various detection methods differ in principle, accuracy, operation complexity, and scope of application [5]. However, in the face of different research purposes and experimental conditions, how to choose the appropriate detection methods has become an important problem faced by researchers [6].

This article aims to systematically review and compare the current mainstream detection methods for lactoferrin iron saturation, including ultraviolet-visible spectrophotometry, saturation analysis, etc. A comprehensive analysis is

conducted from multiple dimensions including detection principles, accuracy, and operational complexity, with the aim of providing a valuable reference for researchers when selecting detection methods. At the same time, we also look forward to the emergence of more innovative detection methods in the future to meet the diverse needs of different fields for the detection of lactoferrin iron saturation.

1. Materials and Methods

1.1 Materials and instruments

Lactoferrin, Australia, 92% purity.

Tv-1810 ultraviolet-visible light detector, Beijing Puxi General Instrument Co., Ltd; BP211D electronic balance, Sartorius company; 25 mm filter.

Reagents such as ferric chloride hexahydrate, triacetic acid (NTA), sodium dihydrogen phosphate monohydrate, sodium hydrogen phosphate decodihydrate, sodium bicarbonate, sodium hydroxide, and sodium chloride were analyzed pure.

1.2 Methods

1.2.1 Saturation analysis (method 1)

1.2.1.1 Experimental principle

Fe^{3+} solution was slowly added to the aqueous lactoferrin solution until lactoferrin reached iron saturation, and a spectrophotometer was used to detect the absorbance value of the solution at 465 nm until the absorbance value no longer changed, that is, lactoferrin reached saturation. Based on this, the Fe^{3+} saturation in lactoferrin samples can be calculated [7].

1.2.1.2 Reagent preparation

1) Prepare solutions containing 10 mmol ferric chloride and 0.1 mol HCl.
2) Prepare a 40 mmol Na-NTA solution: Add 0.764 g of NTA to a 100 mL volumetric flask, using 1 M NaOH to fully dissolve the NTA powder.
3) Prepare solutions containing 5 mmol/L of $FeCl_3$ $6H_2O$ solution and 20 mmol/L of NaNTA solution: Mix 20 mL of (1) and (2) solutions and adjust pH to 5 with 10 M NaOH solution.
4) Prepare 0.1 mol/L $NaHCO_3$ solution.
5) Prepare a 2% (w/w) aqueous solution of lactoferrin.

1.2.1.3 Detection steps

The solution was filtered using a 0.45μm microporous filter prior to use. Pour 1 mL of lactoferrin solution into the cuvette and add 1.5 mL 0.15 mol/L NaCl and 200 μL 0.1 mol/L $NaHCO_3$ solution to read the absorbent value of the solution at 465 nm; When 10μL Fe^{3+} solution was added and fully mixed, the absorbance value at 465 nm was measured, so repeated until the absorbance value no longer changed or decreased substantially.

1.2.1.4 Iron saturation calculation

The ordinate is taken as the absorbance value and the abscissa as the equal amount of trivalent iron ion concentration. The straight line (1) is the light absorption value when lactoferrin tends to be saturated. The line (2) is the line with the initial lactoferrin absorption value as the origin to the absorption value at iron saturation. The initial iron saturation of lactoferrin can be calculated by formula (1).

$$\text{Iron saturation in the sample, } X = \frac{b_1}{b_2} \times 100 \quad (1)$$

where b1 is the initial absorptive value at 465 nm; b_2 is the absorbent value of the solution at saturation.

1.2.2 UV-visible spectrophotometry (method 2)

1.2.2.1 Experimental principle

The absorbance of the 10 mg/mL lactoferrin solution at a mass concentration that reached saturation was 0.48 at 465 nm. The iron saturation in the unknown concentration solution can be estimated accordingly. Before measurement, the solution should be filtered through a 0.45 μm microporous filter membrane, and the measured value at A280 should be between 1.00 and 1.50 after dilution [8].

1.2.2.2 Detection steps

Accurately weigh 0.25g of the sample into a 50 mL beaker, add approximately 20 mL of sodium chloride solution, and place it on a magnetic stirrer to stir until completely dissolved. Then, transfer the solution into a 25 mL volumetric flask,

mix thoroughly, and adjust the volume to the mark, resulting in a 10 mg/mL lactoferrin solution. Transfer 1 mL of the above solution into a 10 mL volumetric flask, dilute it with sodium chloride solution, mix thoroughly, and adjust the volume to the mark, resulting in a 1 mg/mL lactoferrin solution. Then, pass both the 10 mg/mL and 1 mg/mL lactoferrin solutions, along with 20 mL of sodium chloride solution, through a 0.45 μm microporous membrane filter.

Turn on the spectrophotometer to warm up for 20 min. Set the wavelength to 465 nm and adjust the zero point using the filtered sodium chloride solution. The absorbance value of 10 mg/mL of lactoferrin solution measured at 465 nm was recorded as A1. Reset the wavelength to 280 nm and adjust the zero point using the filtered sodium chloride solution. The absorbance value of 1 mg/mL of lactoferrin solution measured at 280 nm was recorded as A2.

1.2.2.3 Iron saturation calculation

The iron saturation of the mass concentration of 10 mg/mL lactoferrin solution is calculated according to the formula (2):

$$\text{Iron saturation in the sample} = \frac{A_1 \times 1.3}{A_2 \times 0.48} \times 100 \quad (2)$$

Where: 1.3 is the absorbance at mass concentration 1mg / mL lactoferrin solution when the solution reaches saturation at 280 nm; A1 is the absorbance value at mass concentration 10mg/mL solution at 465nm; The absorbance value at mass concentration 1 mg/mL lactoferrin solution at 280 nm; 0.48 is the absorbance when the mass concentration of 10mg / mL lactoferrin solution reaches saturation at 465 nm.

1.3 Data processing

Each sample was tested five times, and the results are expressed as the mean ± standard deviation (M ± SD). Independent samples *t*-test using SPSS 26.0 and $P < 0.05$ was considered statistically significant. Utilize the Origin 9.0 software for graphing.

2. Results and Analysis of Lactoferrin Iron Saturation Determination

A total of 10 samples were tested in this experiment, with each sample being tested repeatedly 5 times. The results of iron saturation measured by different detection methods are shown in Table 1. The results show that there are differences in the measurement results of the same sample using different methods. The iron saturation values of the 10 samples measured by Method 1 are generally higher than those measured by Method 2. Among them, the measured values of samples 1 to 9 are lower than those of Method 2, while the measured value of sample 10 is higher than that of Method 2. Moreover, the CV values of Method 1 are generally lower than those of Method 2.

After statistical analysis using SPSS, the results are shown in Figure 1. Except for no significant difference in the detection results between Sample 4, the results of the other samples under different detection methods all showed significant differences ($P < 0.05$), further confirming the variability of results obtained by different detection methods. Therefore, Method 1 tends to give higher iron saturation values overall and exhibits better stability and consistency in repeated measurements.

Table 1. Results of iron saturation of samples determined by different test methods

Sample number	Method 1		Method 2	
	M±SD	CV/%	M±SD	CV/%
1	19.72±0.073	0.37	16.692±0.30	1.79
2	19.350.10±	0.52	16.472±0.44	2.70
3	16.70±0.068	0.41	14.296±0.24	1.71
4	15.66±0.13	0.83	15.26±0.53	3.47
5	18.15±0.40	2.18	15.7±0.39	2.48
6	17.30±0.38	2.22	14.83±0.43	2.93
7	17.76±0.48	2.68	16.49±0.44	2.70
8	16.50±0.42	2.56	14.42±0.35	2.41
9	17.01±0.25	1.47	13.96±0.18	1.30
10	27.04±0.39	1.45	30.00±1.0	3.33

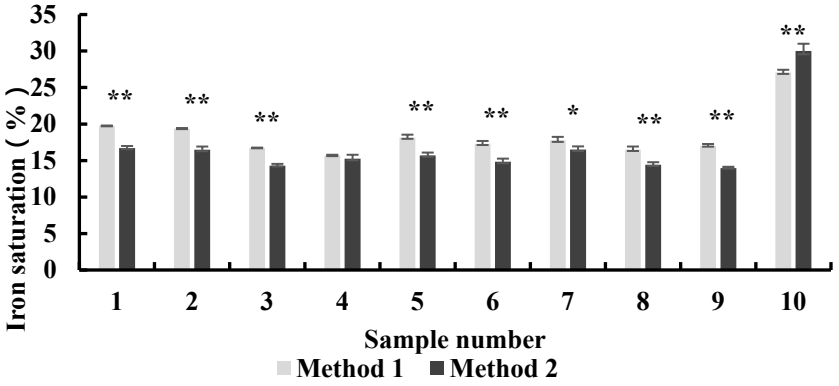

Figure 1. Comparison of results of lactoferrin samples.

3. Conclusion

When measuring the iron saturation of samples, there are significant differences between Method 1 (saturation analysis method) and Method 2 (visible spectrophotometry). These differences are not only reflected in the general level of iron saturation values but also in the stability and consistency (CV values) of the methods. The saturation analysis method generally gives higher iron saturation values than visible spectrophotometry, and the CV values are generally lower, indicating that the saturation analysis method exhibits higher stability and consistency in repeated measurements.

The data fluctuations present in the results may be due to being influenced by certain factors when detecting these samples, such as interference of other components in the sample, loss during sample processing, or sensitivity limitations of the method itself [9]. In order to confirm the causes of these differences, the two methods can be further verified and optimized to improve the accuracy and stability of the determination by calibration with standards, improving sample processing steps, and optimizing instrument conditions. In practice, when choosing which method to use, the purpose of the experiment, sample characteristics, cost-effectiveness, and the actual situation of the laboratory [10]. If the experiment requires high accuracy and stability, and method 1 performs better in this respect, then method 1 can be prioritized. It can also further study the advantages and disadvantages of both methods and explore new methods that combine their advantages to improve the overall performance of iron saturation determination.

References

[1] Ran L, Shi J, Lin Y, et al. Evaluation of the Protective Bioactivity and Molecular Mechanism Verification of Lactoferrin in an Alzheimer's Mouse Model with Ulcerative Enteritis [J]. Journal of Dairy Science, 2024.

[2] Wang Caiyun, Luo Shubo, Yan Xudong, et al. Comparison of methods for lactoferrin iron saturation [J]. Dairy Industry of China, 2015, 43(02): 54-56+64.

[3] Qi Yingjie, Zheng Nan, Wang Jiaqi, et al. Structure-activity effects, detection methods and influencing factors of different iron saturation of lactoferrin in milk [J]. Journal of Animal Nutrition, 2024, 36(06): 3482-3490.

[4] Su Xia, Liu Songyan, Zhu Zichen, et al. Study on the detection method of lactoferrin in fresh milk and dairy products [J]. Livestock and Veterinary Today, 2024, 40(02): 11-13.

[5] Bokkhim H, Tran T, Bansal N, et al. Evaluation of different methods for determination of the iron saturation level in bovine lactoferrin [J]. Food Chemistry, 2014, 152, 121-127.

[6] Ni Dan. Evaluation and study of lactoferrin properties [D]. Inner Mongolia Agricultural University, 2011.

[7] Bengoechea, C., Peinado, I., & McClements, D. J. Formation of protein nanoparticles by controlled heat treatment of lactoferrin: Factors affecting particle characteristics. Food Hydrocolloids, 2011, 25(5), 1354-1360.

[8] Irene F, Matteo D, Benedetta C, et al. Evaluation of hydration with lactoferrin on late-instar; Tenebrio molitor; larvae performance and functional properties of obtained meal [J]. Italian Journal of Animal Science, 2023, 22(1): 982-994.

[9] Nika O, Timeja P P, Albin K, et al. Stability-Indicating Analytical Approach for Stability Evaluation of Lactoferrin [J]. Pharmaceutics, 2021, 13(7): 1065-1065.

[10] Lavanya M, Rao A R, Kumar A K, et al. Evaluation of the reproductive toxicity of antiretroviral drug loaded lactoferrin nanoparticles [J]. Systems Biology in Reproductive Medicine, 2019, 65(3): 205-213.