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A New Quantitative Determination Method of Acetoin

Hui Xu[1§], Yanjun Tian[1§], Kunfu Zhu[2], Shanshan Wang[1], Lei Zhu[2], Qiangzhi He[1], Wenjing Li[3] and Jianjun Liu[1*]

[1]Key Laboratory of Food and Fermentation Engineering of Shandong Province, Shandong Food Ferment Industry Research & Design Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250014, China
[2]Shandong Zhushi Pharmaceutical Group Co., Ltd, Heze 274300, China
[3]Food and Drug College, Shandong Institute of Commercial Technology, Jinan 250100, China
**Corresponding author*
[§]These authors contributed equally to this work.

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A B S T R A C T

To obtain a new quantitative determination method of Acetoin for reducing the cost of testing and the workload in the process of strain breeding. In an alkaline environment, using 3,5-dinitrosalicylic acid with strong oxidizing properties to azeotrope with Acetoin, 3,5-dinitrosalicylic acid is reduced to 3-amino-5-nitrosalicylic acid and 3-amino-5-nitrosalicylic acid are red-brown substances. The content of Acetoin can be obtained by detecting the absorbance at 540 nm. This new quantitative determination method of Acetoin is a simple, rapid and low-cost method for quantitative determination of Acetoin.

Introduction

Acetoin is chemically named 3-hydroxy-2-butanone. It is an α-hydroxy ketone with a molecular formula of C4H8O2 and a molecular weight of 88.12. The second and third carbons in the molecule are asymmetric carbon atoms and have two chirality. Isomer, its chemical structure is:

The boiling point of Acetoin is 148°C and the melting point is 15 °C. It is self-igniting, soluble in propylene glycol and ethanol, miscible in water, and slightly soluble in ether (Xu *et al.*, 2011; Tian *et al.*, 2016; Fan *et al.*, 2013). Acetoin has a pleasant creamy fragrance and is a commonly used spice variety in the world. The American Food and Extraction Association (FEMA) has approved its use in foods. The CAS number is 513-86-0 (Xu *et al.*, 2011; Tian *et al.*, 2016). The FEMA safety number is 2008. In addition, Acetoin is a 4C platform compound, which is widely used in many industries (Zhang *et al.*, 2017; Yu *et al.*, 2008; Zhang *et al.*, 2001). In 2004, the US Department of Energy listed Acetoin as one of the 30 priority development and utilization platform compounds (Ji *et al.*, 2008; Christen and López-Munguia, 1994; Hummel *et al.*,

1992; Defaveri *et al.*, 2003; Xiao *et al.*, 2007). At this stage, the methods for quantitative detection of Acetoin in fermentation broth mainly include creatine colorimetry, gas chromatography (GC) and liquid chromatography (HPLC).

However, the creatine colorimetry method has a shortcoming. The detection reagents must be prepared and used immediately, which is time-consuming and labor-intensive and often leads to waste of reagents. Although the two methods of gas phase and liquid phase have high accuracy and accuracy, they have high requirements on equipment, and the detection speed is slow, time-consuming and laborious, and is not suitable for the detection of large quantities of samples. Therefore, the establishment of a rapid quantitative detection method for Acetoin can not only reduce the cost of detection, but also significantly reduce the workload of product detection in the process of strain breeding.

Acetoin belongs to α-hydroxy ketone, with active chemical properties and typical α-hydroxy aldehydes and ketones. Therefore, through comprehensive analysis, in an alkaline environment, we choose 3,5-dinitrosalicylic acid with strong oxidizing properties to azeotrope with Acetoin, and 3,5-dinitrosalicylic acid is reduced It is 3-amino-5-nitrosalicylic acid. 3-amino-5-nitrosalicylic acid is a brown-red substance. Within a certain range, Acetoin is directly proportional to the color of the brown-red substance. Detect the absorbance of the brown-red substance at the wavelength of 540 nm, and the content of Acetoin can be calculated through the standard curve. At present, there is no report on the method of quantitative determination of Acetoin using 3,5-dinitrosalicylic acid and Acetoin azeotropically.

Materials and Methods

Preparation of Acetoin standard solution

100 mg of Acetoin standard sample was transfered to a 100 mL volumetric flask, distilled water to the mark, shake well, keep in the refrigerator for later use.

Preparation of 3,5-Dinitrosalicylic acid reagent

Add 6.3 g of 3,5-dinitrosalicylic acid and 262 mL of 2 M NaOH solution to 500 mL of hot aqueous solution containing 185 g of potassium sodium tartrate, then add 5 g of crystalline phenol and 5 g of sodium sulfite, and stir to dissolve. After cooling, add distilled water to make the volume to 1000 mL, and store in a brown bottle for later use.

Preparation of Acetoin standard curve

Take seven 25 mL colorimetric tubes with stoppers and add reagents according to Table 1.

Results and Discussion

Acetoin standard curve

Shake each tube well, heat it in a boiling water bath for 5 min, take it out and cool it to room temperature with tap water, add distilled water to make the volume up to 25 mL, and mix well. At a wavelength of 540 nm, use tube 0 as a control, measure the absorbance of tubes 1-6, and draw a standard curve.

As shown in Fig. 1, the absorbance of the solution has a linear relationship with the concentration of Acetoin. The linear equation of the curve is $y=16.585x+0.0114$. The concentration of Acetoin can be obtained by using the above formula.

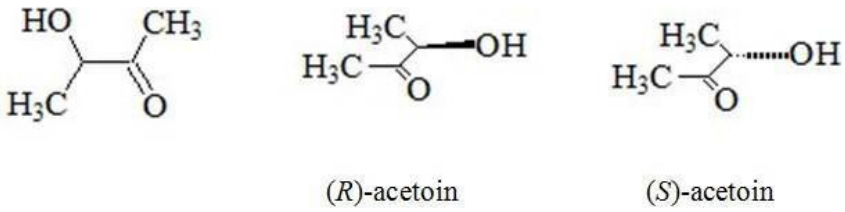
Linear range verification

In order to verify the concentration of Acetoin and the linear range of absorbance, on the basis of drawing the standard curve of Acetoin, prepare several standard solutions of higher concentration of Acetoin, measure the absorbance value, verify the linear range of the Acetoin standard curve.

It can be seen from Fig. 2 that when the concentration of Acetoin exceeds 0.08 mg/mL, the

absorbance value is not within the linear range of the standard curve of Acetoin so when determining the concentration of Acetoin fermentation broth, try to Dilute the fermentation broth to below 0.08 mg/mL to ensure that the absorbance is within the linear range of the Acetoin standard curve.

Fig.1



(*R*)-acetoin (*S*)-acetoin

Fig.2 Standard curve of Acetoin

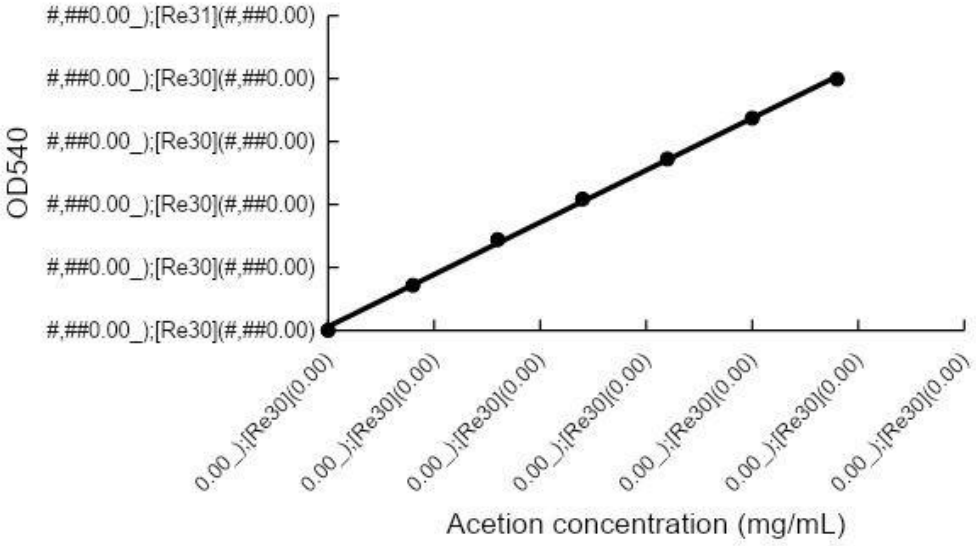


Fig.3 Linear range verification curve of Acetoin

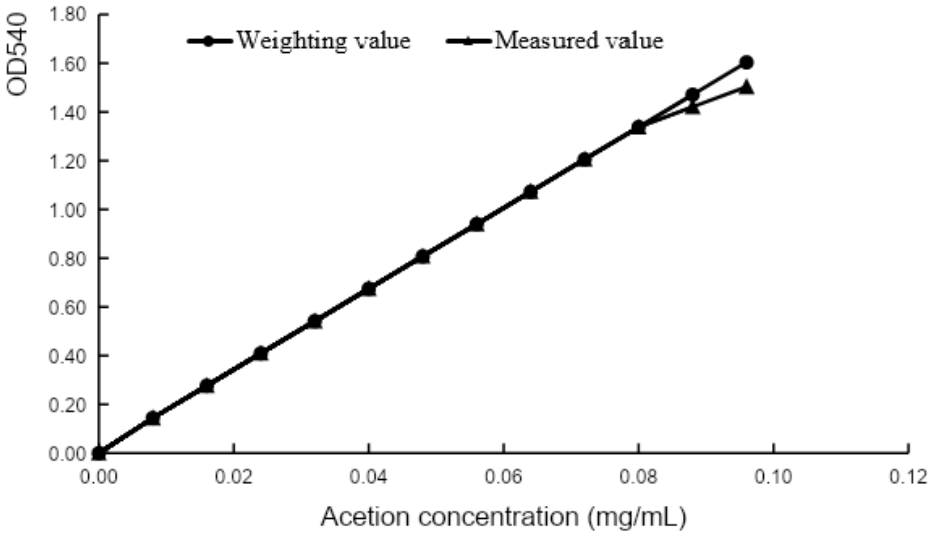


Fig.4 Gas chromatogram of fermentation broth tested

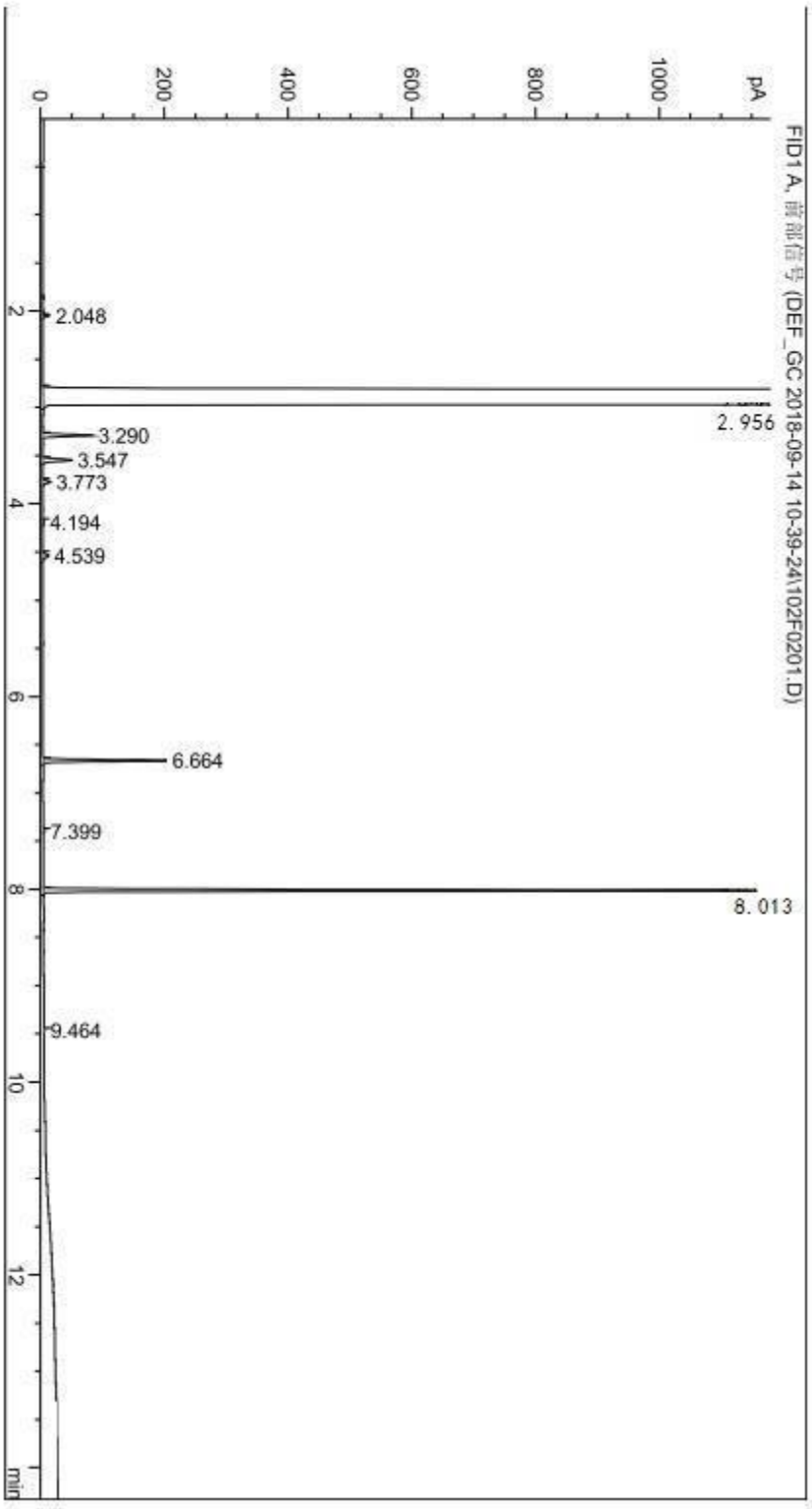


Table.1 Preparation of 3,5-Dinitrosalicylic acid reagent

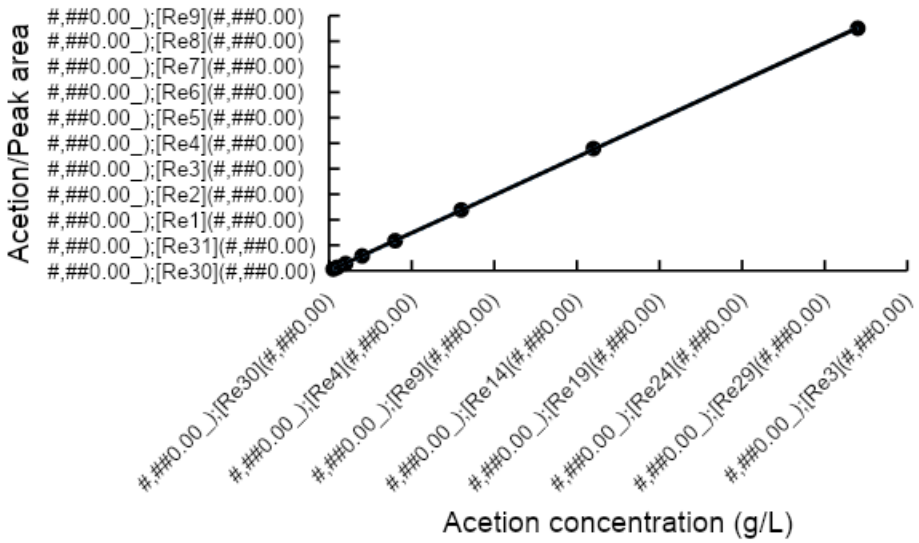
Tube No.	0 (mL)	1 (mL)	2 (mL)	3 (mL)	4 (mL)	5 (mL)	6 (mL)
A	0	0.2	0.4	0.6	0.8	1.0	**1.2**
B	2.0	1.8	1.6	1.4	1.2	1.0	**0.8**
C	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**	**1.5**

A: Acetoin standard solution; B: Distilled water; C: 3,5-Dinitrosalicylic acid.

Table.2 Detection results of Acetoin concentration with different methods

Method	1st Acetoin (g/L)	2nd Acetoin (g/L)	3rd Acetoin (g/L)
New method	15.66	29.67	**44.63**
GC	15.23	30.12	**45.12**
Relative error	**2.82**	**1.49**	**1.11**

Fig.5 Standard curve for determination of Acetoin concentration by GC



Method verification

In order to verify the feasibility of this method, a strain producing Acetoin was selected to ferment 3 times for 72 h, the glucose was exhausted, and the fermentation liquid was obtained by centrifugation. After the fermentation liquid was diluted 100 times with distilled water, 3,5-dinitro was added. The salicylic acid reagent is heated and boiled for 5 min.

After the reaction is completed, the absorbance of the solution is measured, substitute the detected absorbance value into the linear equation to obtain the concentration of Acetoin in the fermentation broth.

At the same time, gas chromatography was used to determine the concentration of Acetoin in the fermentation broth. The gas chromatogram is shown in Fig. 3, and the concentration of Acetoin was determined according to Figs. 3 and 4. The detection results of this method and gas chromatography are shown in Table 2. It can be seen that the relative error of Acetoin in the three fermentation broths

measured by the new method and gas chromatography simultaneously is very small.

The new quantitative determination method of Acetoin is fast in detection speed, time-consuming and laborious, and is suitable for the detection of large quantities of samples. It can not only reduce the cost of detection, but also significantly reduce the workload of product detection in the process of strain breeding.

Acknowledgments

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Conflict of interest

All authors declare that they have no conflict of interest.

References

Christen, P., López-Munguia, A. 1994. Enzymes and food flavor: a review. Food Biotechnol 8: 167-190.

Defaveri, D., Torre, P., Molinari, F. 2003. Carbon material balances and bioenergetics of 2,3-butanediol bio-oxidation by *Acetobacter hansenii*. Enzyme Microb Technol 33: 708-719.

Fan, Y. X., Tian, Y. J., Zhao, X. Y., Zhang, J. X., Liu, J. J. 2013. Isolation of acetoin-producing bacillus strains from Japanese traditional food—natto. Prep Biochem Biotech 43: 551–564.

Hummel, W., Kula, M. R., Boermann, F. Microbiologically prepared diacetyl reductase. U.S. Patent 5164314, Nov 17, 1992.

Ji, X. J., He, H., Du, J. 2008. Progress in the synthesis and application of acetoin. Mod Chem Ind 28: 81.

Tian, Y. J., Xu, H., Liu, J. J., Chen, W., Sun, W. T., Chen, Y. Q. 2016. Construction of acetoin high-producing *Bacillus subtilis* strain. Biotechnol Biotec Eq 30: 700-705.

Xiao, Z. J., Liu, P. H., Qin, J. Y. 2007. Statistical optimization of medium components for enhanced acetoin production from molasses and soybean meal hydrolysate. Appl Microbiol Biotechnol 74: 61-68.

Xu, H., Jia, S. R., Liu, J. J. 2011. Development of a mutant strain of *Bacillus subtilis* showing enhanced production of acetoin. Afr J Biotechnol 10: 779-788.

Yu, T. M., Wang, Y. H., Yang, B. 2008. Study on the preparation of natural milk scent base by combination of microbial fermentation and enzymatic method. Food Ferment Ind 34:153-157.

Zhang, J. J., Zhao, X. Y., Zhang, J. X., Zhao, C., Liu, J. J., Tian, Y. J., Yang, L. P. 2017. Effect of deletion of 2,3-butanediol dehydrogenase gene (*bdh*A) on acetoin production of *Bacillus subtilis*. Prep Biochem Biotech 47: 761-767.

Zhang, X. Z., Zeng, C. Y., Ren, X. G. 2001. Current status and prospects of synthetic study of acetyl couples. Jiangsu Chem Ind 4: 29-31.

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