Hindawi
Journal of Food Quality
Volume 2020, Article ID 8846214, 7 pages
https://doi.org/10.1155/2020/8846214

Research Article

Food Safety Risk Assessment of γ-Butyrolactone Transformation into Dangerous γ-Hydroxybutyric Acid in Beverages by Quantitative ^{13}C-NMR Technique

Shaoming Jin,[1] **Xiao Ning**,[1] **Jin Cao**,[1] **and Yaonan Wang**[2]

[1]*National Institutes for Food and Drug Control, 100050 Beijing, China*
[2]*Core Facilities Centre, Capital Medical University, 100069 Beijing, China*

Correspondence should be addressed to Yaonan Wang; yang1039@126.com

Received 29 April 2020; Revised 11 July 2020; Accepted 21 July 2020; Published 1 August 2020

Academic Editor: Luis Patarata

Food safety remains a matter of great concern in most countries and the composition in food is crucial to food safety. It is very important to make sense of the quality and change of food ingredients. In this research, the change of γ-butyrolactone (GBL), one kind of food additive in beverage, had been evaluated by nuclear magnetic resonance (NMR) technique. The ^{1}H-NMR results of seven beverages covering various kinds with spiked GBL indicated that GBL was transformed into dangerous γ-hydroxybutyric acid (GHB) in six popular beverages under certain conditions which could happen during transportation and storage. Further results of quantitative ^{13}C-NMR showed that pH and temperature were two key factors affecting the transforming degree of GBL to GHB. Lower pH and higher temperature will increase the degree of transformation. GHB was a neurotransmitter on the chemical control list, which was absolutely forbidden to be added to food. This nondestructive NMR detecting technology which did not need the complex pretreatment method to directly determine food ingredients can be useful for identifying the risk of food safety from the changes of food composition during transport and storage.

1. Introduction

With the prosperity of food industry, the ingredients of food become more complex. Safety incidents related to food ingredients emerge endlessly, among which food additives have become the most common causes of food safety incidents and a major public concern [1–3]. As one kind of important food ingredients, food additives are widely used in bread, beverage, meat products, and so on [4]. In recent years, the overall level of food quality and safety has been steadily improved due to the increasingly strict supervision of the use of food additives [5, 6].

In addition to the monitoring of food adulteration and illegal addition, the change of food ingredients including legal additives also needs attention [7, 8]. The existing researches on the changes of food ingredients are mainly divided into two categories. One is the changes of food ingredients caused by microbial pollution, the research focal point of which is on microorganisms; Moreira's team found that the deterioration of fallow deer and goat meat was in proportion to the number of microbiological counts by the measurement of meats via Fourier transform infrared spectrometer (FTIR) [9]; researchers from china got the similar findings, even under vacuum and low temperature; the microbial community in foods reached its highest diversity after two weeks in refrigerated storage via microbial deoxyribonucleic acid (DNA) sequencing [10]. The other is the changes caused by chemical reaction of food composition, oxidation, or the process of loss of electrons, hydrogen abstraction, or flow of unpaired electrons, which may occur in all the chemical constituents of muscle foods, including lipids, muscle pigments, structural proteins, and enzymes [11]. Studies undertaken so far were mostly focusing on whole food; very little was found in the literature on the change of food additives. Since there is an urgent need to address the safety problems caused by prolonged use of

food additives [12, 13], it is prior to make clear whether the food additives are stable in foods.

GBL is a conventional food additive, used to increase the aroma of food. It belongs to lactone in terms of chemical structure, which is stable in neutral aqueous solution but not when the pH value of solution changes and hydrolysis will occur [14, 15]. GHB, the hydrolyzed product of GBL, is a neurotransmitter with strong central nervous system depressant effect; it is on the list of controlled chemicals and it is absolutely forbidden to be used for food [16, 17]. In order to enrich the taste of modern drinks, some acidic or alkaline substances will be added, which will lead to the deviation of pH value from 7. Once GBL is added to these drinks, it is possible to hydrolyze to GHB. Elliott and Burgess [18] found that naturally occurring GHB and GBL were detected in those beverages involving the fermentation of white and particularly red grapes via NMR. They were concerned about whether there was one or two of the two substances in the beverage, but the source of the two substances was not very clear. Lesar et al. [19] developed a method for the quantitation of GHB and GBL spiked into beverages using a new water suppression technique; this method allowed for the direct identification and quantitation of both compounds in all beverages except red and white wine, where small interferences prevented accurate quantitation. However, they were concerned about whether the ethanol in beverages will affect the quantification, also ignoring the conversion of GBL to GHB in beverages, so the quantification in some beverages may not be accurate. All of the above studies showed that NMR was an excellent method to study the composition changes in complex samples [8, 20, 21].

In this paper, GBL was spiked into seven beverages involving coffee drink, orange juice, soda water, fruit vinegar drink, energy drink, green tea drink, and cola; after extremely simple pretreatment procedure, the hydrolysis of GBL in beverages was identified. Firstly, since ^{1}H-NMR was a fast, nondestructive, sensitive method for the detection of chemical compounds and the change of reaction in liquid state [22–24], the presence of GHB in simulate acidic conditions and these beverages was confirmed by ^{1}H-NMR with solvent presaturation; then considering the accuracy of quantification is affected as the overlap and interference of signals in ^{1}H-NMR, the quantity of GHB in these beverages was determined by quantitative ^{13}C-NMR [25–27]. The degree of hydrolysis of GBL was determined by the amount of GHB measured, so as to evaluate the safety risk of adding GBL to the beverages.

2. Materials and Methods

2.1. Chemicals and Reagents. Formic acid for analysis was purchased from Merck Co. (Darmstadt, Germany). Ammonium hydroxide was purchased from Beijing chemical plant (Beijing, China). All the beverages were purchased from the supermarket (Yonghui superstores, Beijing, China). Yaha mocha coffee was produced by Uni-President China Holdings Ltd.; NFC orange juice was produced by Nongfu Spring Co., Ltd.; Watson's soda water drink was produced by Guangzhou Watson's Co., Ltd.; Red Bull vitamin energy drink was produced by Red Bull Vitamin Beverage Co., Ltd.; green tea drink was produced by Uni-President China Holdings Ltd.; fruit vinegar drink was produced by Henan Huiduozi Beverage Co., Ltd.; cola was produced by Pepsi Co.

2.2. Instrumentation and Parameters. The NMR instrument was AVANCE III 500 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a broadband observe (BBO) probe. The water bath was HH-600 digital display constant temperature water bath pot (Jiejia, Anqing, China). The NMR tubes and deuteroxide were purchased from Tenglong weibo Co. Ltd (Qingdao, China). The ^{1}H-NMR experiment was performed using the method mentioned before by Lesar et al. [19]. Briefly, the parameter was set as follows: the acquisition time was 1.28 s, the 90-degree pulse width was 8.78 μs, and the relaxation delay was 2.0 s and 32,768 time domain points. As to the acquisition of quantitative ^{13}C-NMR, inverse gated decoupling sequence was applied to suppress nuclear Overhauser effect (NOE) of proton [28] and the relaxation delay time was optimized. Other acquisition parameters were as follows: time domain 32 K; dummy scans, 4; number of scans, 512; acquisition time, 0.69 s; spectral width, 220 ppm (27500 Hz); fid resolution, 0.83 Hz; and total acquisition time, 50 min.

2.3. Sample Preparation. A 0.2% aqueous solution of formic acid was used to simulate acidic condition and a 1% aqueous ammonia solution was used to simulate alkaline condition. For coffee and orange juice, the precipitate was removed by centrifugation prior to sample preparation. Other samples were used for analysis without any processing. Two series of samples were parallel prepared, one containing GBL and one without GBL. For samples with GBL, 440 μL sample and 10 μL GBL were mixed together and then 50 μL deuteroxide was added into the mixture. For sample without GBL, 450 μL of sample and 50 μL deuteroxide were mixed together. The homogeneous mixed liquid was added into the NMR tube for test. In order to simulate the real environment of beverage during transportation and storage, the samples were treated at different temperature to evaluate the effect of temperature on hydrolysis rate. For the ^{1}H-NMR determination, the sample was pretreatment at 35°C overnight, which was very common in summer. For the quantification of GHB in beverage, the sample was pretreatment at temperature higher than 35°C; in order to speed up the hydrolysis reaction and improve efficiency, the thermal condition was set to four temperatures, which were 35°C, 45°C, 55°C, and 65°C, which could be reached in extreme cases. One kind of sample was made four copies in parallel and treated for two hours at four temperatures, respectively. The NMR tubes were directly put into the water bath which had reached the target temperature preset. After two hours of treatment, the NMR tubes were taken out from water bath and cooling down to room temperature and then put the tube into NMR spectrometer to acquire carbon spectrum.

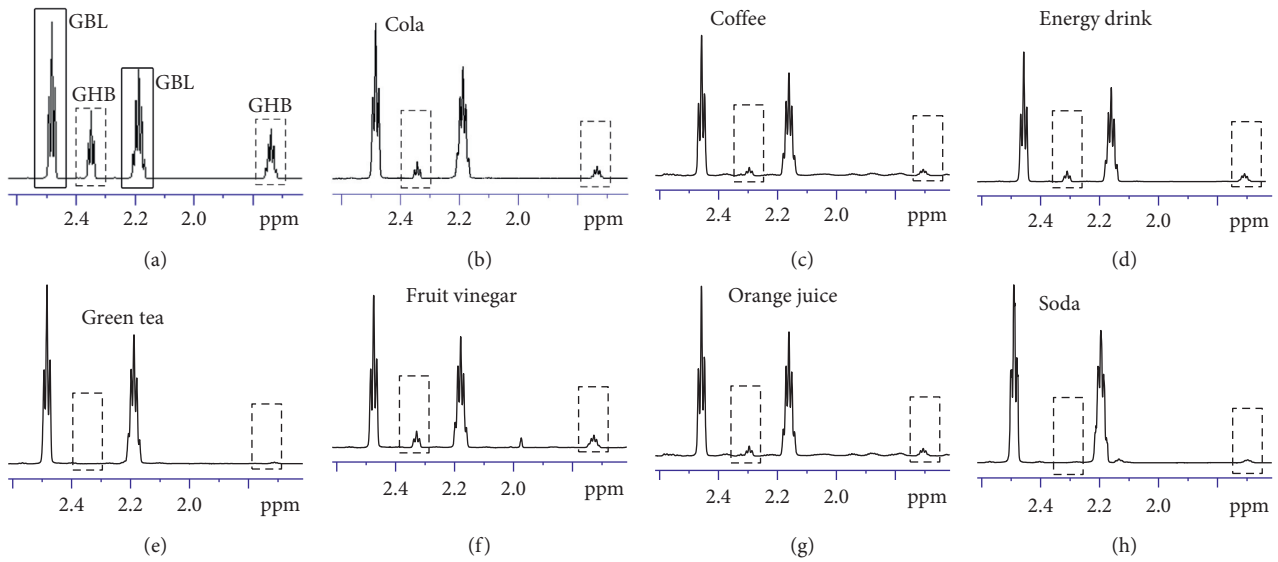

Figure 1: The 1H-NMR results of GBL spiked into simulated acidic solution (a) and other seven popular beverages (b–h). The signals in solid wireframe in (a) were attributed to GBL and signals in dotted wireframe in all figures were attributed to GHB.

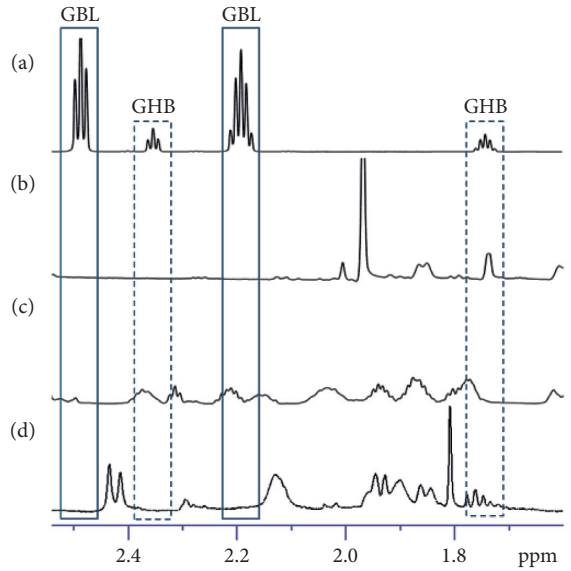

Figure 2: The local enlarged 1H-NMR spectrum of GBL spiked into 0.2% aqueous solution of formic acid (a), fruit vinegar drink (b), orange juice (c), and coffee drink (d).

3. Results and Discussion

3.1. Hydrolysis of GBL in Beverages. The ^{1}H-NMR results of GBL spiked into 0.2% aqueous solution of formic acid and seven beverages are shown in Figure 1 and the signals of GBL and GHB were separated with different wireframes. From Figure 1(a), the stronger signals in solid wireframe belonged to GBL; the weaker signals in dotted wireframe belonged to GHB, which was consistent with previous study [19]. To confirm whether GHB appeared in beverage samples, the signal areas of GHB in all beverage spectrums were highlighted with dotted wireframe. The GHB signals were observed in all seven beverages other than green tea. The signals in coffee drink and soda water were weaker compared to other beverages but the signal-to-noise ratios were all above 50. Considering the complex matrix of coffee drink, the result of Figure 1(c) needed further confirmation.

In order to confirm that the signal of beverage matrix would not interfere with the identification of GHB from the results of ^{1}H-NMR, the ^{1}H-NMR spectra of beverages without GBL were acquired and are shown in Figure 2. Three beverages contained substances that overlap with GHB signals; fruit vinegar drink, orange juice, and coffee drink all have signals at the same locations attributed to the methylene of GHB. These results indicated that it was necessary to combine the two signals of methylene to determine whether

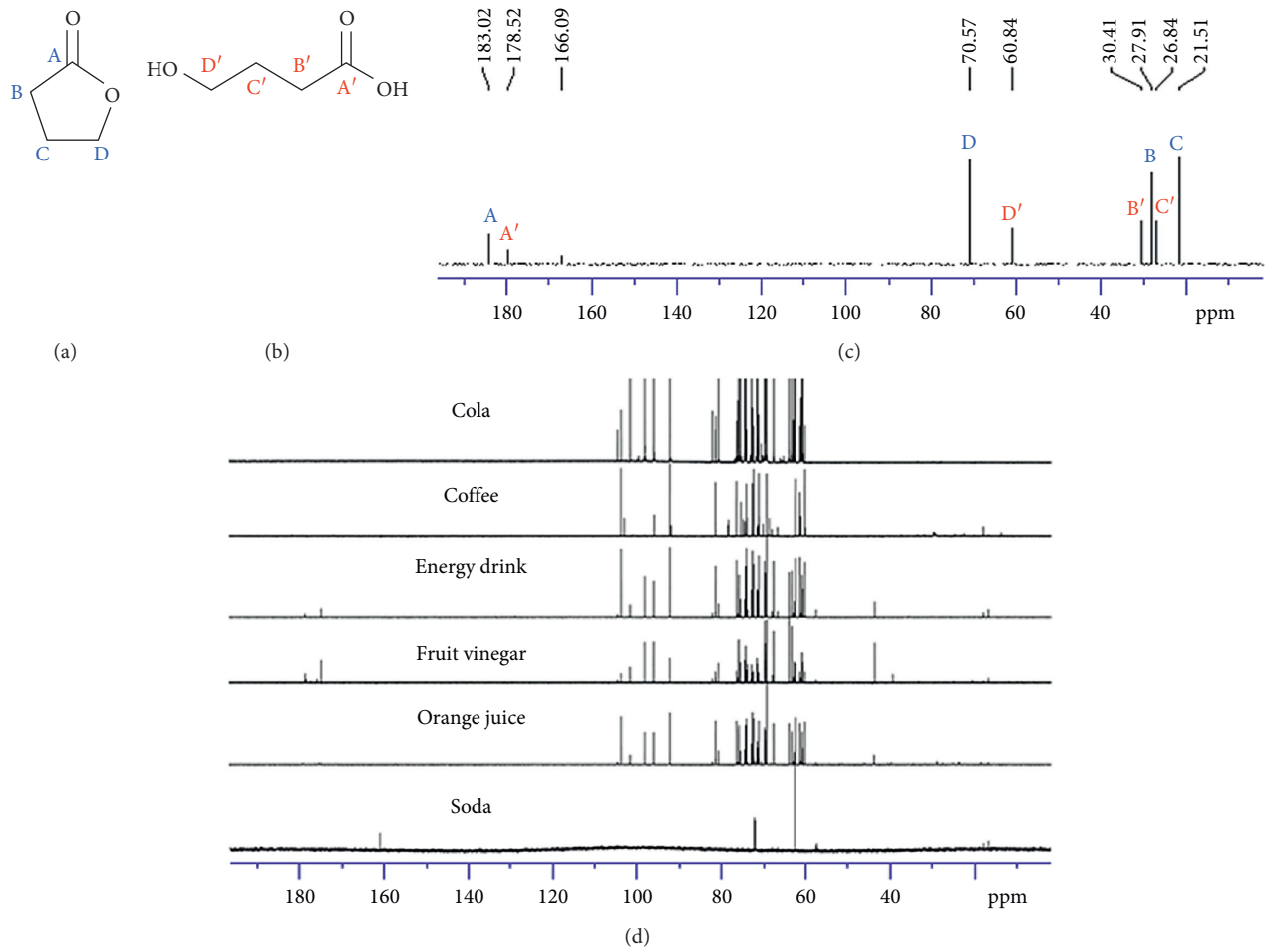

FIGURE 3: The chemical structure of GBL (a) and GHB (b). The 13C-NMR spectrum of GBL spiked into 0.2% aqueous solution of formic acid (c) and beverages without GBL (d).

GHB was generated. If it was used for quantitative analysis, the hydrogen spectrum was not accurate enough.

3.2. ^{13}C-NMR of GBL and GHB. Since the H-NMR signals of GHB and GBL were easily influenced by complex matrices, quantitative ^{13}C-NMR was used to quantify the degree of GBL hydrolysis. Before quantitative ^{13}C-NMR measurement, ordinary ^{13}C-NMR was implemented to identify the appropriate carbon atom for quantification. GBL was a lactone with a five-membered cyclic structure, which is shown in Figure 3(a); there were three secondary and one quaternary carbon atoms in the molecule. GHB was a straight-chain carboxylic acid containing four carbon atoms with a hydroxyl group modification at the gamma carbon atom, which is shown in Figure 3(b). The ^{13}C-NMR spectrum of GBL spiked into 0.2% aqueous solution of formic acid is shown in Figure 3(c). The signals had been labeled in different colors to indicate different compound. Signal at 166.09 ppm indicated the carbon atom in formic acid. The ^{13}C-NMR of beverages without GBL is shown in Figure 3(d). Most of the carbon signals of the materials in these samples were concentrated in the high field region, and it was clear and definite that there was no signal in the region higher than 180 ppm and lower than 40 ppm, which meant the signal of 183.02 ppm attributed to the quaternary carbon atom and signals of 27.91 ppm and 21.51 ppm attributed to the secondary carbon atoms were all appropriate for quantification of GBL without any interference. Similarly, the signal of 178.52 ppm attributed to the quaternary carbon atom and the signal of 26.84 ppm and 21.51 ppm attributed to the secondary carbon atoms were all appropriate for quantification of GHB.

3.3. Optimization of Relaxation Delay Time of Quantitative ^{13}C-NMR. The relaxation delay time ($D1$) is a key parameter affecting quantitative results; different $D1$ resulted in different signal intensities [29]. Although as a rule of thumb pulse repetition time is kept at least equal to five times the value of T1 of the slowest relaxing nuclei, here three carbon atoms with different type could be used for quantification, and different $D1$ was set to confirm the shortest but enough time for determining the hydrolysis degree of GBL. As shown in Figure 4, the intensity ratios of four carbon atoms plotted against different $D1$ were reported. As mentioned above, except d' and d, carbon atoms (γ carbon atoms of GBL and GHB) had signals overlap with those matrix of the

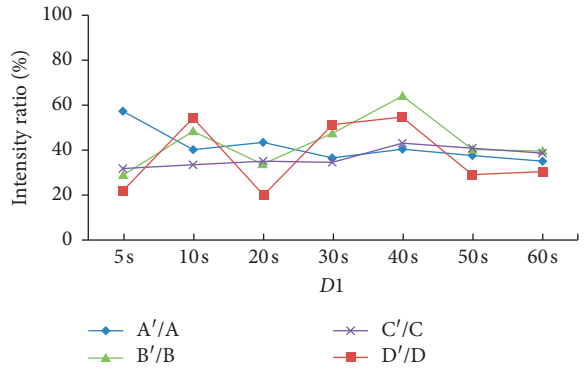

Figure 4: The intensity ratio of different carbon atoms plotted against different $D1$ time points.

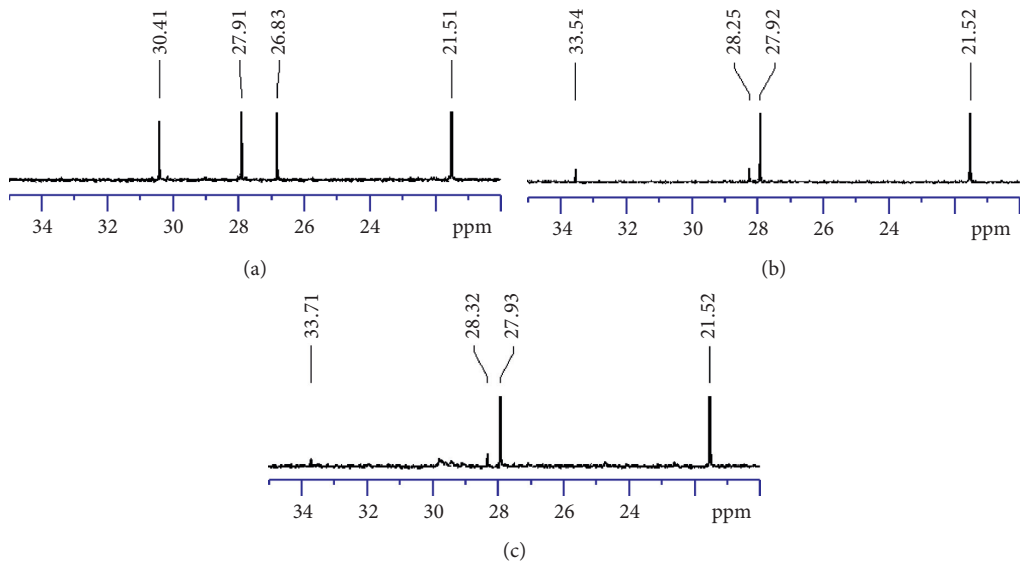

Figure 5: The partially enlarged 13C-NMR of GBL spiked into 0.2% aqueous solution of formic acid (a), GBL spiked into soda water (b), and GBL spiked into coffee drink (c).

beverages; other three kinds of carbon atoms were evaluated with different $D1$. With the extension of $D1$ time, such as 50 s and 60 s, the ratio of carbon atom intensity tends to be similar, which was consistent with the traditional rule [30, 31]. However, for carbon atom c' and c, the intensity ratio at different $D1$ time did not display dramatic differences. Even if $D1$ was set to 5 s, almost similar intensity ratio of c'/c (the β carbon atom of GHB and GBL) could be obtained as $D1$ was set at 60 s. In the follow-up association research, $D1$ was set to 5 s and the intensity ratio of c'/c was used to identify the hydrolysis degree of GBL in beverages.

3.4. Downfield of Chemical Shift of β Carbon Atom of GHB in Coffee Drink and Soda Water. The chemical shift was closely related to the electron cloud density on the carbon atom. The electron cloud density of carbon atom in carboxylic acid and carboxylate was different, so the chemical shift was also inconsistent [32–34]. In this research, GHB was one kind of carboxylic acid; when sodium salt and other food additives were simultaneously present in beverages, they would form carboxylate with GHB. As shown in Figure 5, the α and β carbon atom of GHB in coffee drink and soda water were all downfield to low field; this suggested that these two beverages contained sodium salt and formed carboxylate with GHB.

For soda water, the change of chemical shift only affected the quantitative results; the risk that GBL could be hydrolyzed was already recognized by proton spectrum. For coffee drink, the GBL hydrolyzed or not was not confirmed by ^{1}H-NMR; it was important to identify that the forming of carboxylate resulted in the change of chemical shift, and the risk of GBL hydrolysis in coffee did exist.

3.5. Hydrolysis Degree of GBL in Beverages. Quantitative ^{13}C-NMR was used to determine the amount of GHB in beverages after pretreatment under certain conditions simulated real transport and storage. As temperature was one key factor influencing the change of food composition [35–37], four grades of temperature were set to evaluate the hydrolysis of GBL in different beverages. The intensity ratio of

Table 1: The intensity ratio of GHB/GBL in beverages after pretreatment under different temperature.

Temperature (°C)	Beverages type					
	Cola	Coffee	Energy drink	Fruit vinegar	Orange juice	Soda
35	0.048	—	—	—	—	0.042
45	0.132	—	—	0.008	—	0.052
55	0.24	0.01	0.005	0.05	0.004	0.088
65	0.3	0.049	0.117	0.145	0.062	0.109

c'/c in different beverages after pretreatment at different temperature is shown in Table 1.

The hydrolysis results of GBL in beverages roughly divided into three levels of high, middle, and low; each level had two kinds of drinks. The same trend was shown within each level, and hydrolysis degree was inversely proportional to pH value. At the first high degree level, the hydrolysis degree in cola (pH = 2.67) was higher than in soda water (pH = 5.23). At the second middle degree level, the hydrolysis degree in fruit vinegar (pH = 3.32) was higher than in energy drink (pH = 5.92). At the third low degree level, the hydrolysis degree in orange juice (pH = 3.52) was higher than in coffee drink (pH = 6.34). In addition to the effect of pH on the degree of hydrolysis, there was another similar trend in all beverages; the degree of hydrolysis was directly proportional to the temperature. That was to say, at a high temperature, the degree of hydrolysis was also high, and beverages that need to be stored in cold storage needed special attention.

4. Conclusion

The results of this research directly show that one common food additive GBL can hydrolyze to form dangerous GHB in most popular drinks under ordinary transport and storage conditions, especially in carbonated drinks like cola and soda water. Beverages with low pH and pretreatment at high temperature can increase the extent of GBL hydrolysis. This is a risk point of food safety because GHB is a neurotransmitter with strong central nervous system depressant effect. NMR technology is a mature technology that can conveniently, quickly, and nondestructively detect changes in substances, which is very meaningful for food safety research. It should be noted that food safety risks may occur at all stages including manufacture, transportation, and storage. Food regulators need to reassess the risks of adding GBL to beverages and foods to determine if GBL is still suitable as a food additive. Foods that require low temperature storage must strictly adhere to low temperature conditions during transportation and storage. Whether other food additives have similar risks also requires more in-depth research. Food safety is so important that the research of food ingredients must be taken seriously.

Data Availability

All data included in this study are available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was supported by the special fund for project of the National Medical Products Administration (Grant no. 1010071645002).

References

[1] G. Han and Y. Liu, "Does information pattern affect risk perception of food safety? A national survey in China," *International Journal of Environmental Research and Public Health*, vol. 15, no. 9, p. 1935, 2018.

[2] C. Liao, X. Zhou, and D. Zhao, "An augmented risk information seeking model: perceived food safety risk related to food recalls," *International Journal of Environmental Research and Public Health*, vol. 15, no. 9, p. 1800, 2018.

[3] A. M. Hamdan, M. M. Al-Gayyar, M. E. E. Shams et al., "Thymoquinone therapy remediates elevated brain tissue inflammatory mediators induced by chronic administration of food preservatives," *Scientific Reports*, vol. 9, no. 1, p. 7026, 2019.

[4] D. Pandir, "DNA damage in human germ cell exposed to the some food additives in vitro," *Cytotechnology*, vol. 68, no. 4, pp. 725–733, 2016.

[5] L. Wu, Q. Zhang, L. Shan, and Z. Chen, "Identifying critical factors influencing the use of additives by food enterprises in China," *Food Control*, vol. 31, no. 2, pp. 425–432, 2013.

[6] L. Wu, Y. Zhong, L. Shan, and W. Qin, "Public risk perception of food additives and food scares. The case in Suzhou, China," *Appetite*, vol. 70, pp. 90–98, 2013.

[7] L. Manning and J. M. Soon, "Developing systems to control food adulteration," *Food Policy*, vol. 49, pp. 23–32, 2014.

[8] S. Cai, Y. Zhang, F. Xia, G. Shen, and J. Feng, "An expert system based on 1H NMR spectroscopy for quality evaluation and adulteration identification of edible oils," *Journal of Food Composition and Analysis*, vol. 84, Article ID 103316, 2019.

[9] M. J. P. Moreira, A. C. Silva, J. M. M. M. D. Almeida, and C. Saraiva, "Characterization of deterioration of fallow deer and goat meat using microbial and mid infrared spectroscopy in tandem with chemometrics," *Food Packaging and Shelf Life*, vol. 15, pp. 169–180, 2018.

[10] Y. Zhang, Y. Yao, L. Gao, Z. Wang, and B. Xu, "Characterization of a microbial community developing during refrigerated storage of vacuum packed Yao meat, a Chinese traditional food," *LWT*, vol. 90, pp. 562–569, 2018.

[11] J. Aalhus and M. Dugan, "Spoilage, factors affecting | oxidative and enzymatic," in *Encyclopedia of Meat Sciences*, M. Dikeman and C. Devine, Eds., pp. 394–400, Academic Press, Cambridge, MA, USA, 2nd edition, 2014.

[12] N. Kumar, A. Singh, D. K. Sharma, and K. Kishore, "Chapter 3-toxicity of food additives," in *Food Safety and Human Health*, R. L. Singh and S. Mondal, Eds., pp. 67–98, Academic Press, Cambridge, MA, USA, 2019.

[13] T. Jansen, L. Claassen, I. V. Kamp, and D. R. M. Timmermans, ""All chemical substances are harmful." public appraisal of uncertain risks of food additives and contaminants," *Food and Chemical Toxicology*, vol. 136, Article ID 110959, 2020.

[14] X.-L. Yin, H.-L. Wu, H.-W. Gu et al., "Second-order calibration method applied to process three-way excitation–emission-kinetic fluorescence data: a novel tool for real-time quantitative analysis of the lactone hydrolysis of irinotecan in human plasma," *Chemometrics and Intelligent Laboratory Systems*, vol. 146, pp. 447–456, 2015.

[15] Y. Yin, J. G. P. Binner, M. J. Hey, and J. R. Mitchell, "Hydrolysis of carboxylic lactones in alumina slurries," *Journal of the European Ceramic Society*, vol. 26, no. 7, pp. 1171–1177, 2006.

[16] G. P. Galloway, S. L. Frederick-Osborne, R. Seymour, S. E. Contini, and D. E. Smith, "Abuse and therapeutic potential of gamma-hydroxybutyric acid," *Alcohol*, vol. 20, no. 3, pp. 263–269, 2000.

[17] E. R. Ringel, "Gamma-hydroxybutyric acid," *The New England Journal of Medicine*, vol. 353, no. 15, pp. 1632-1633, 2005.

[18] S. Elliott and V. Burgess, "The presence of gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) in alcoholic and non-alcoholic beverages," *Forensic Science International*, vol. 151, no. 2-3, pp. 289–292, 2005.

[19] C. T. Lesar, J. Decatur, E. Lukasiewicz, and E. Champeil, "Report on the analysis of common beverages spiked with gamma-hydroxybutyric acid (GHB) and gamma-butyrolactone (GBL) using NMR and the PURGE solvent-suppression technique," *Forensic Science International*, vol. 212, no. 1–3, pp. e40–e45, 2011.

[20] B. Ripper, C. R. Kaiser, and D. Perrone, "Use of NMR techniques to investigate the changes on the chemical composition of coffee melanoidins," *Journal of Food Composition and Analysis*, vol. 87, Article ID 103399, 2020.

[21] D. Paniagua-Vega, N. Cavazos-Rocha, A. A. Huerta-Heredia et al., "A validated NMR method for the quantitative determination of rebaudioside A in commercial sweeteners," *Journal of Food Composition and Analysis*, vol. 79, pp. 134–142, 2019.

[22] A. Brächer, R. Behrens, E. V. Harbou, and H. Hasse, "Application of a new micro-reactor 1 H NMR probe head for quantitative analysis of fast esterification reactions," *Chemical Engineering Journal*, vol. 306, pp. 413–421, 2016.

[23] C.-H. Wu, J.-S. Jeng, J.-L. Chia, and S. Ding, "Multi-nuclear liquid state NMR investigation of the effects of pH and addition of polyethyleneglycol on the long-term hydrolysis and condensation of tetraethoxysilane," *Journal of Colloid and Interface Science*, vol. 353, no. 1, pp. 124–130, 2011.

[24] M. Leutzsch, A. J. Sederman, L. F. Gladden, and M. D. Mantle, "In situ reaction monitoring in heterogeneous catalysts by a benchtop NMR spectrometer," *Magnetic Resonance Imaging*, vol. 56, pp. 138–143, 2019.

[25] W. Wollinger, J. L. N. Fernandes, L. H. K. Q. Júnior, B. C. Garrido, and F. R. D. A. Neto, "Improving quantitative 13C NMR performance by an adiabatic scheme," *Microchemical Journal*, vol. 140, pp. 167–175, 2018.

[26] L. Marchetti, V. Brighenti, M. Rossi, J. Sperlea, F. Pellati, and D. Bertelli, "Use of 13C-qNMR spectroscopy for the analysis of non-psychoactive cannabinoids in fibre-type cannabis sativa L. (Hemp)," *Molecules*, vol. 24, no. 6, p. 1138, 2019.

[27] C. Simmler, J. G. Napolitano, J. B. McAlpine, S.-N. Chen, and G. F. Pauli, "Universal quantitative NMR analysis of complex natural samples," *Current Opinion in Biotechnology*, vol. 25, pp. 51–59, 2014.

[28] G. Singh, A. V. Kothari, and V. K. Gupta, "Triad sequence determination of ethylene–propylene copolymers–application of quantitative 13C NMR," *Polymer Testing*, vol. 28, no. 5, pp. 475–479, 2009.

[29] T. A. Darwish, N. R. Yepuri, P. J. Holden, and M. James, "Quantitative analysis of deuterium using the isotopic effect on quaternary 13C NMR chemical shifts," *Analytica Chimica Acta*, vol. 927, pp. 89–98, 2016.

[30] E. Caytan, G. S. Remaud, E. Tenailleau, and S. Akoka, "Precise and accurate quantitative 13C NMR with reduced experimental time," *Talanta*, vol. 71, no. 3, pp. 1016–1021, 2007.

[31] R. Sacchi, A. Paduano, N. Caporaso, G. Picariello, R. Romano, and F. Addeo, "Assessment of milk fat content in fat blends by 13C NMR spectroscopy analysis of butyrate," *Food Control*, vol. 91, pp. 231–236, 2018.

[32] N. DiDonato and P. G. Hatcher, "Alicyclic carboxylic acids in soil humic acid as detected with ultrahigh resolution mass spectrometry and multi-dimensional NMR," *Organic Geochemistry*, vol. 112, pp. 33–46, 2017.

[33] G. Świderski, S. Wojtulewski, M. Kalinowska, R. Świsłocka, and W. Lewandowski, "Effect of alkali metal ions on the pyrrole and pyridine π-electron systems in pyrrole-2-carboxylate and pyridine-2-carboxylate molecules: FT-IR, FT-Raman, NMR and theoretical studies," *Journal of Molecular Structure*, vol. 993, no. 1–3, pp. 448–458, 2011.

[34] X. Xiao, J. Liang, J. Xie, X. Liu, D. Zhu, and Y. Dong, "Organotin (IV) carboxylates based on 2-(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl) acetic acid: syntheses, crystal structures, luminescent properties and antitumor activities," *Journal of Molecular Structure*, vol. 1146, pp. 233–241, 2017.

[35] A. Roccato, M. Uyttendaele, and J.-M. Membre, "Analysis of domestic refrigerator temperatures and home storage time distributions for shelf-life studies and food safety risk assessment," *Food Research International*, vol. 96, pp. 171–181, 2017.

[36] S. Im, M.-K. Lee, Y.-M. Yun, S.-K. Cho, and D.-H. Kim, "Effect of storage time and temperature on hydrogen fermentation of food waste," *International Journal of Hydrogen Energy*, vol. 45, no. 6, pp. 3769–3775, 2020.

[37] M. Blankart, C. Oellig, S. Averweg, W. Schwack, and J. Hinrichs, "Effect of storage at high temperature on chemical (composition) and techno-functional characteristics of E471 food emulsifiers applied to aerosol whipping cream," *Journal of Food Engineering*, vol. 277, Article ID 109882, 2020.