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Simultaneous Analysis of Preservatives in Beverages Samples Using High Pressure Liquid Chromatography

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Abstract: The development of ways to test the quantities of preservatives in beverages is required, since they are often included in these drinks. It is important to keep an eye on the amount of preservatives used in drinks since several research have shown that they may cause a variety of disorders. The researchers in this study set out to find out how much sodium benzoate and potassium sorbate were in various drinks sampled from the city of Medan. They also wanted to make sure their analytical approach was sound.This study used a reversed-phase HPLC technique using an Eclipse plus C18 column measuring 250 x 4.6 mm and 5 µm, a phosphate buffer mobile phase with a pH of 5, a 70:30 ratio of metanol, and a flow rate of 1.0 ml/minute. The linearity test passed with a coefficient of determination (R2) of 0.99998 - 0.99999, as shown in the method validation results. A range of 0.2541-0.31101 mg/kg was observed for the LOD values. There was an intermediate accuracy of 0.24% to 0.28%, a percent RSD of the repetability values ranging from 0.21% to 0.24%, and a LOQ from 0.8469 to 1,0338 mg/kg. An average of 99.95% to 104.52% recovery was the outcome. Beverage sweetener determination using the described approach has been a success.

Keywords: Beverages, HPLC, Preservative.

Introduction

In Indonesia, sales of soft drinks (ready to drink) continues to experience rapid growth from year to year, as seen from the increasing number of soft drink products in various brand on the market. Data obtained from the ministry of industry, the growth of the beverage and food industry during the period 2015-2019 averaged around 8,16%. Food and beverage industry is still able to grow positively by 1.58% in 2020 amidst the pandemic condition (Nasution, 2022).

The use of soft drinks has become ubiquitous and is now considered an integral component of many people's daily routines (Lim,et al, 2018). Excessive consumption of sugary drinks is associated with an increased risk of obesity and other health problems. Insulin sensitivity and the prevalence of chronic diseases such as obesity and hypertension are influenced by the preservative content in soft drinks (Hwang,et al, 2020).

In Indonesia, sales of ready-to-drink soft drinks continue to increase every year, which can be seen from the increase in various brands of soft drink products on the market. According to information obtained from the ministry of industry, between 2015 and 2019, the food and beverage sector grew by an average of around 8.16%. Despite the pandemic scenario, according to Nasution, 2022 and Hwang, 2020 estimates that the food and beverage industry will continue to grow positively by 1.58% in 2020 found that the prevalence of chronic diseases including obesity, type 2 diabetes, and hypertension is increasing partly due to changes in eating habits, one of which is the increasing use of soft drinks.

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There is research related to analysis methods of beverages using HPLC ; Yazdanfar,et al, 2023; Suprianto,et al, 2018; 2018; Prayuda, et al, 2023; Jankulovska, et al, 2016; Asci, et al, 2016 are some of the studies investigating the use of high-performance liquid chromatography (HPLC) for simultaneous analysis of preservatives.

The purpose of this research is to develop a method for determining preservative (potassium sorbate and sodium benzoate) simultaneously in order to perform the testing procedure more quickly, correctly, effectively, and with scientific confidence, it is important to establish a simultaneous analytical approach to detect the amount of preservatives (potassium sorbate and sodium benzoate) using an HPLC instrument.

Method

Materials The materials consisted of soft drink samples and standard solutions of sodium benzoate and potassium sorbate (BPFI). Aquabidest, phosphoric acid (Merck), potassium dihydrogen phosphate, dicalium hydrogen phosphate, and HPLC grade methanol. Tools This investigation included various instruments, including a pH meter, analytical balance, UV 1800 spectrophotometer, a set of Eclipse plus C18 columns with a particle size of 5 µm and dimensions of 250 x 4.6 mm, and a set of HPLC equipment including a PDA detector (Shimadzu LD 20 AD). The necessary equipment were: digital sonicator from Mettler-Toledo, vacuum pump, 0.45 polytetrafluoroethylene (PTFE) membrane filter, mobile phase container, pipettes with volumes of 1, 2, 3, 5, and 10 ml, measuring cups with volumes of 10, 50, and 100 ml, micropipettes with volumes of 100-1000 µl from Eppendorf, and various other glassware.

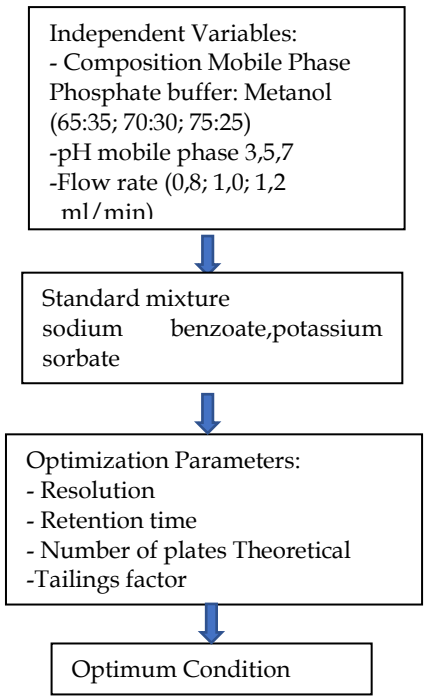
Stages of Research

The research was conducted at the Medan Food and Drug Monitoring Center Laboratory from September 2023 to February 2024. By using experimental methodology to determine the effect of the interrelationship of the independent variable and the dependent variable, this research uses descriptive and experimental approaches. In its most basic form, an experimental study explains how one variable (x) affects another variable (y) through a chain reaction. The sodium benzoate and potassium sorbate content levels of soft drinks were investigated using descriptive research methodology.

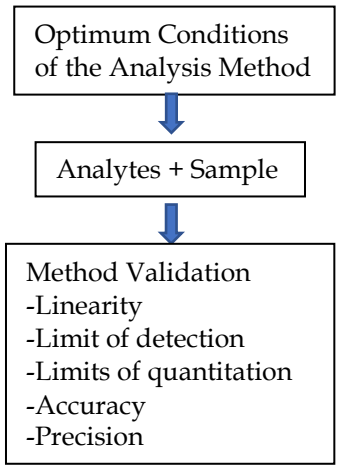
Research Procedure

The research framework can be seen in the work scheme below:

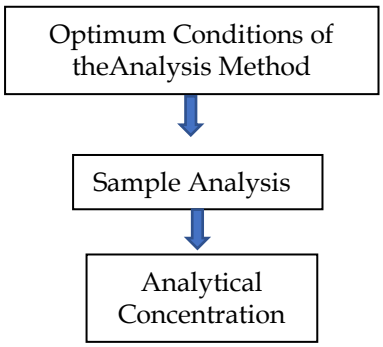
1. Optimization of Simultaneous Analysis Method for Several Types of Sweeteners and Preservatives in Soft Drink Products using High Performance Liquid Chromatography Method



2. Validation of Simultaneous Analysis Method for Several Types of Preservatives in Soft Drink Products by High Performance Liquid Chromatography Method



3. Simultaneous Analysis of Several Types of Preservatives in Soft drink Products using High Performance Liquid Chromatography Method



Sampling of Soft Drinks

This study surveyed soft drink manufacturers in Medan City to find out whether the ingredients contained preservatives potassium sorbate and sodium benzoate. Data on the content of analytes added to the samples were collected by selecting five different brands of soft drinks. The selected samples had to be undamaged and within the expiry date. After that, five different brands of soft drinks containing the analytes were selected for testing. These drinks were then classified into A, B, C, D, and E. Using sonication, the mixture was stirred for a few minutes after adding distilled water into a 50 ml measuring flask containing about 2 grams of soft drink. Next, transfer 2.0 ml of the sample solution into a 10 ml volumetric flask using a pipette. Then add distilled water and dissolve until the line is reached. After the test solution passed through the 0.45 µm membrane syringe filter, 20 µl was injected into the autosampler vial. After that, we draw a linear regression line equation Y = bX + a by plotting the area against the concentration of each analyte. Furthermore, the regression equation of each identified analyte was used to determine the analyte levels.

Wavelength Determination

Wavelength determination standard curves for benzoate and sorbate were prepared by first determining the wavelength of analysis for each analyte. Twenty microliters of each 10 ppm standard solution was injected after filtering through a 0.45 µm PTFE filter using sonication. Using a UV spectrophotometer, the absorbance was measured in the wavelength range of 190 to 300 nm. Subsequent experimental parameters will be determined using the spectra obtained.

Optimum Conditions of Mobile Phase Variation, Mobile Phase pH and Flow Rate

To optimize the process and achieve effective compound separation, one can adjust the flow rate, mobile phase composition and pH, as well as other relevant parameters. All characteristics, such as tailing factor, resolution, retention duration, and theoretical plate count, must conform to specifications.

Method Validation

Once the analytical technique is optimized, key parameters including linearity, limit of detection, limit of quantification, accuracy, and precision are tested to ensure the findings are reliable.

Linearity of Standard

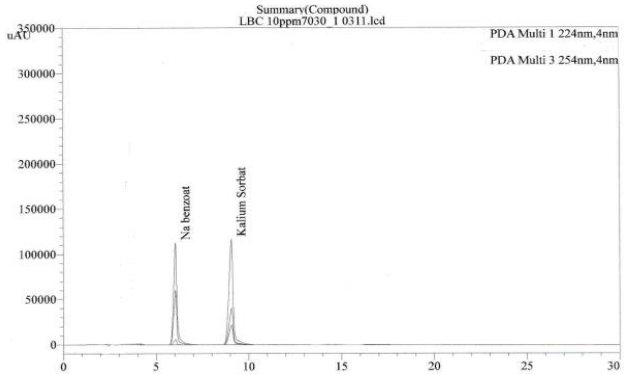
After passing through a 0.45 µm PTFE needle filter, the standard solution from the circuit was disinfected for 15 minutes. The HPLC apparatus was injected with 20 µL of reference solution. The chromatogram obtained was analyzed to determine the area of each analyte, then the linear regression equation Y = aX + b was calculated for each analyte and the regression correlation value (r) was also calculated. According to Astuti, 2019, a method is said to have good linearity if the value of $r > 0.99$ or $R^2 > 0.997$.

Accuracy and Precision

In this study, repeatability and intermediate precision were tested six times in a row. For both daily (intermediate precision) and multi-day (repeatability test) sample preparation, the presence of random error can be determined using the repeatability test (Department of Health RI, 2020). The percentage of relative standard deviation is used to indicate precision. The level of accuracy is assessed by the small value of RSD. The applicability of the system was tested precisely by injecting the 10 ppm reference solution six times. A placebo (simulated) recovery approach was used to measure the percentage recovery. This method involves adding several analytes to a sample containing no analytes (placebo), and then analyzing the combination (Susanti & Dachriyanus, 2017). By capturing the absorption spectra of standard solutions of benzoate and sorbate and other analytical components, one can ascertain the analytical wavelengths. (Susanti & Dachriyanus, 2017).

Result and Discussion

Standard solutions of sodium benzoate and potassium sorbate were prepared to determine the analytical wavelengths using absorption spectroscopy, UV spectrophotometer. From the full spectrum analysis, it was found that the longest wavelengths of all the analytes were in the 190-300 nanometer range. The ideal absorption of these two analytes has been measured using three different detector wavelengths, namely at 224 nm and 254 nm wavelengths (Yazdanfar, 2023); (Jankulovska, et al, 2016); (Suprianto, et al, 2018); Mubarok, 2021; Prayuda, et al, 2023; Younes, 2019).



Description: LBC = Mixed standard solution of both analytes

Figure 1. Chromatogram of each analyte in the optimal system by HPLC

Table 1. Wavelength optimization of each standard by HPLC

Analytes	Wavelengths(nm)	Retention time	Resolution	Tailing factor
Sodium Benzoate	224 nm	6.054	9.223	1.074
Potassium Sorbate	254 nm	9.076	8.158	0.913

A mobile phase composition ratio of 70:30 methanol:pH 5 phosphate buffer was determined to provide optimal analytical conditions. It fulfills the criteria and outperforms other phosphate buffers in terms of resolution, theoretical limit, retention duration, and tailing factor.

Linearity

Linearity is the ability of a method to give findings that are directly proportional to analyte concentration within a certain range. It was determined whether the analytes potassium sorbate, sodium benzoate were linear using six sets of standard solutions. See Figure 1 for linearity graphs of the five analytes. (Selen and Stromgren, 2019).

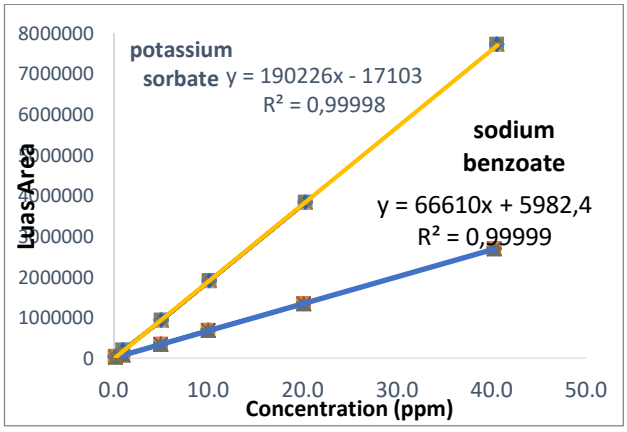


Figure 2. Linearity Curve

Sodium benzoate and potassium sorbate are analytes that have a linear relationship between the instrument response and the analyte concentration and the r value is close to 1, as shown in the linearity graph above. The AOAC guideline states that the correlation coefficient should be more than 0.99, which means that the standard curve shows linearity when injected three times with concentration variations of 80%, 100%, and 120%. The results achieved are in accordance with the 90%-107% recovery criteria suggested by AOAC (Department of Health RI, 2020; AOAC, 2016). This indicates a high level of accuracy of this research strategy. This demonstrates the high level of accuracy of this research strategy. excellent to show that the measurement value corresponds to the true value.

% Recovery

Two grams of analyte-free placebo was combined with conventional sodium benzoate and potassium sorbate at 80%, 100%, and 120% concentrations for this investigation. Afterwards, the mixture was injected three times. (AOAC, 2016; Selen and Stromgren, 2019). The findings obtained were in the 90% to 107% recovery range. This proves that the study approach is quite accurate in revealing how well the measured value matches the actual value.

Table 2. % Recovery values of each analite

Treatment Accuracy	% Recovery	
	Sodium Benzoate	Potassium Sorbate
80%	103,9111	102,5913
100%	104,5269	99,0770
120%	103,2868	100,6326

The recovery value is not significantly different from the sodium benzoate recovery rate of 98.80 µg/ml to 102.25 µg/ml found in the study by Asci et al. (2016) and the study by (Magomya, 2020) with an accuracy rate of 94.54 – 97.25% mean recovery was recorded for benzoate and 92.7 – 96.82% for sorbate. Whereas the research from (Tarigan, 2023) obtained a recovery value of 91.88% for sodium benzoate and 91.27% for potassium sorbate.

Precision

The precision test of this study included six replicate injections of a combination of sample and analyte solutions at a single concentration (100%). Six independent replicates of the repeatability and intermediate precision tests were performed as part of this study. By comparing the results of samples prepared on the same day (repeatability test) and another day (intermediate precision), it can be ascertained whether there is random error resulting from sample preparation. According to (Susanti &

Dachriyanus, 2017). The level of accuracy is assessed by the small value of RSD. Injecting a standard solution of 10 specific molecules (ppm) six times enables precise system compatibility testing (Stromgren and Stromgren, 2019).

Table 3. Precision values of each standard compound

Precision	Standard	
	Sodium Benzoate	Potassium Sorbate
System suitability test (RT ; Area)	0.21 ; 0.03	0.21; 0.07
Repeatability test	0.55	0.49
Precision test	0.24	0.28

Limit of Detection (LD) & Limit of Quantitation (LK)

For the purpose of precision testing of this study, we injected a combination of sample solutions including additional analytes six times at a single concentration level (100%) to ensure uniformity. The study included six replicates of repeatability and intermediate precision tests. When processing samples either on the same day (reliability test) or on different days (intermediate precision), the presence of random errors can be detected. According to (Susanti & Dachriyanus, 2017), the level of accuracy increases as the RSD value decreases. A reference solution with a concentration of 10 ppm was injected six times to ensure accuracy in evaluating the application of the system (Selen and Stromgren, 2019).

Table 4. Limit of detection and limit of quantitation

Parameters	Sodium Benzoate	Potassium Sorbate
LOD (ug/ml)	0.2075	0.2532
LOQ (ug/ml)	0.6915	0.8441

Results showed that the limit of detection varied between 0.2075 and 0.2532 ug/ml, while the limit of quantification varied between 0.6915 and 0,8441 ug/ml, for both analytes. The LOD and LOQ values are not significantly different from the research conducted by (Javanmardi, 2014). LOD and LOQ were for benzoate 0.1 and 0.5 µg mL-1 , respectively, and for sorbate 0.08 and 0.3 µg mL-1, respectively. The results showed that benzoic and sorbic acid widely occur in soft drink products in Iran.

Analyte Levels in Samples

Table 5. Levels of natirum benzoate and potassium sorbate in soft drinks

Soft Drinks Concentration of analyte found in the sample	Analyte	Concentration of Analyte in the simple	Maximum use limit (BPOM RI no 11 of 2019)
A	Sodium Benzoate	278,7873	400 mg/kg
	Potassium Sorbate	374,0663	1000 mg/kg
B	Sodium Benzoate	289,3666	400mg/kg
	Potassium Sorbate	219,6823	1000mg/kg
C	Potassium Sorbate	221,8645	1000mg/kg
D	Potassium Sorbate	151,1080	1000mg/kg
E	Sodium Benzoate	77,1457	400 mg/kg
	Potassium Sorbate	207,7445	1000mg/kg

We tested three different soft drink brands offered in Medan for the amount of sodium benzoate and potassium sorbate. To perform the calculation, the area values were plugged into the standard curve of the regression equation obtained through the linearity test. The findings showed that sample B had the highest concentration of sodium benzoate, at 289.36 mg/kg while the lowest was found in sample E, at 77.14 mg/kg. And findings from previous studies also showed quite similar results. The highest potassium sorbate level was found in sample A at 374.07 mg/kg, while the lowest level was found in sample E at 151.11 mg/kg.

The concentration of sample analytes is within the range specified in BPOM rule no. 11 of 2019 which states that it is safe for public use. From the chromatogram, it can be seen that the analytes in the sample are well separated and can be identified even though there are other material components and this indicates selective research Food and Drug Administration, 2019).

The value obtained from this research are still within the range of (Javanmardi, 2014). The result values are not significantly different. Benzoic acid was detected in 50 (92.5%) of the samples ranging from 3.5 to 1520 µg mL-1, while for sorbic acid 29 (50.3%) samples presented the preservative in a range of 0.8 and 2305 µg mL-1.The value of potassium sorbate is not much different from the study by (Tarigan, 2023) which is 292.14 mg/kg and (Hayun, 2004) research where the benzoic acid valued at 206,81 mg/kg.

Conclusion

Analytical conditions using a C18 column (240 x 4 mm) and methanol phosphate-buffered mobile phase can be used to determine the levels of sodium benzoate

and potassium sorbate simultaneously using the high-performance liquid chromatography (HPLC) method. The analytical conditions of the study were phosphate-buffered pH 4 (70:30); 1.0 ml/min, and 224 nm and 254 nm PDA detectors. The levels of analytes in soft drinks were obtained between 77.14 and 374.07 mg/kg. This amount still meets the standard requirements set by the government.

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Author Contributions
The authors listed in this article, have read and agree to the published version of the manuscript.

Conflicts of Interest
The authors declare no conflict of interest

References

AOAC. (2016). Appendix F : Guidelines for Standard Method Performance Requirements. In *AOAC Official Methods of Analysis*.

Asci,B., S.D., Zor and O.A.Donmez. (2016). Development and Validation of HPLC Method for the Simultaneous Determination of Five Food Additives and Caffeine in Soft Drinks. *International Journal of Analytical Chemistry 9*(10). https://doi.org/10.1007/s12161-016-0458-x

Astuti, E.J., R.F.N. Ilham J. Rahman. (2019). Validation method for determining sodium benzoate in fruit juice drinks in Malang. *Jurnal Farmasi Dan Ilmu Kesehatan*, *4*(1), 19–23.

Food and Drug Administration. (2019). Indonesian Food and Drug Authority. *Bpom*, *11*, 1–16.

Department of Health RI, 2020. *Farmakope Indonesia Edisi V*.

Hayun, Y. Harahap dan C.n. Aziza. (2004). Penetapan Kadar Sakarin, Asam Benzoat, Asam Sorbat, Kofeina dan Aspartam di dalam Beberapa Minuman Ringan Bersoda Secara Kromatografi Cair Kinerja Tinggi. *Majalah Ilmu Kefarmasian*.

Hwang, S.B., S. Park, G.R. Jin, J. H. Jung, H.J. Park, S.H. Lee, S. Shin and B.H. Lee. (2020). Trends in beverage consumption and related demographic factors and obesity among korean children and adolescents. *Nutrients*, *12*(9), 1-13. https://doi.org/10.3390/nu12092651

Jankulovska, M.S., L.V. Markovska, B.P. Ilievska dan S. Trpkovska. (2016). High-Performance Liquid Chromatography Method for Determination of Preservatives in Beverages. *Journal of Agricultural, Food, and Environmental Sciences*, *3*.

Javanmardi, F., M. Nemati, M. Ansarin and S.R. Arefhosseini.. (2014). Benzoic and sorbic acid in soft drink, milk,ketchup sauce and bread by dispersive liquid, liquid microextraction coupled by HPLC. *Food Additives & Contaminants: Part B*, 20.

Lim, H., H.J.Lee, R. Choue and Y. Wang. (2018). Trends in Fast-Food and Sugar-Sweetened BeverageConsumption and Their Association with Social Environmental Status in South Korea. *Journal of the Academy of Nutrition and Dietetics*, *118*(7), 1228-1236.e1.https://doi.org/10.1016/j.jand.2017.08.001

Magomya, A. M., G.G. Yebpella, U.C. Okpaegbe, O.J. Oko and S.B. Gambo. (2020). Analysis and Health Risk Assessment of Sodium Benzoate. *International Journal of Pharmacy and Chemistry*, 54.

Mubarok, F. (2021). HPLC Prinsip dan Cara Kerja. *Jurnal Farmasi Industri*, *June*, 2–3.

Nasution, A. (2022). Peningkatan Kinerja Industri Makanan dan Minuman Melalui Transformasi Digital di Indonesia. *Trending: Jurnal Manajemen Dan Ekonomi*, *1*(1), 165–176. https://doi.org/10.30640/trending.v1i1.493

Prayuda, E.M., F.F. Hasanah, R. Valensia, N.D. Rahmawati dan M.R. Utami. (2023). *Methods of Analysis of Sodium Benzoate in Food and Beverages*, *6*(2), 508–514.

Stromgren, S and Selen. A. (2019). *Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products 3rd Edition*.

Suprianto, E.D.L., Putra dan S.M. Sinaga. (2018). Optimization of Volume Void and Wavelengths at Simultaneous Determination Method Development of Sweeteners, Preservatives and Dyes by UFLC. *International Journal of ChemTech Research*, *10*(1), 89–97. http://sphinxsai.com/2017/ch_vol10_no1/1/(89-97)V10N1CT.pdf

Susanti, M dan Dachriyanus. (2017). Kromatografi Cair Kinerja Tinggi. *Lembaga Pengembangan Teknologi Informasi dan Komunikasi (LPTIK) Universitas Andalas*.

Tarigan, E.C. (2023). Penetapan kadar natrium benzoat, kalium sorbat Asesulfam K dalam minuman berperisa secara KCKT di PT Saraswanti Indo Genetech.

Yazdanfar N, Manafi L, Ebrahiminejad B, Mazaheri Y, Sadighara P, Basaran B, Mohamadi S. (2023). Evaluation of Sodium Benzoate and Potassium Sorbate Preservative Concentrations in Different Sauce Samples in Urmia, Iran. *J Food Prot.* 86(8):100118. https://doi.org/10.1016/j.jfp.2023.100118.

Younes, M., G. Aquilina, L. Castle, K.H.Engel, P. Fowler, M.J. Fernandez, P. Furst, R. Gurtler, U. G. Remy, T. Husoy, W. Mennes, P. Moldeus, A. Oskarsson, R. Shah, D. Wolfle, C. Lambre and I. W. Berendsen. (2019). Opinion on the follow-up of the re-evaluation of sorbic acid (E200) and potassium sorbate (E202) as food additives. *EFSA Journal*, *17*(3). https://doi.org/10.2903/j.efsa.2019.5625