UDC 664:543.635: 57.084
DOI: 10.21323/2618–9771–2019–2–4–31–33
Original scientific paper

DEVELOPING THE METHODS OF FOOD PRESERVATIVES EXTRACTION FROM COMPLEX MATRICES FOR BIOASSAY PURPOSE

Artem V. Samoilov*, Natalia M. Suraeva, Sergey V. Koptsev, Sergey V. Glazkov

All-Russian Scientific Research Institute of Technology of Preservation —
Branch of the V. M. Gorbatov Federal Research Center for Food Systems of RAS, Vidnoye, Moscow Region, Russia

KEY WORDS:
sorbic acid, benzoic acid, extraction, bioassay, the Allium test, preserved vegetable products

ABSTRACT
The presence of food additives in food products may be associated with the risk of their toxic effects on human body. Therefore, the study of approaches to testing their safety seems to be a particularly urgent task. The aim of this study was to determine the conditions for extracting food preservatives from the samples of preserved pureed vegetables for further bioassay of the extract obtained in the Allium test. Onion roots were used as a test object in this method. Two extraction methods of benzoic and sorbic acids added to pureed vegetables have been developed. Distilled water and acetone were used as extracting solutions. The extraction efficiency was evaluated on Shimadzu Prominence LC-20 liquid chromatograph (Japan) in the ultraviolet range, wavelength 235 nm (benzoic acid), 285 nm (sorbic acid). According to the results of studies using both water and acetone as extractants, the degree of preservatives extraction was approximately the same and quite high. In the quantitative calculation of the preservatives content in pureed vegetables, the value of the correction factor was 0.8. However, due to certain production characteristics of this product, i.e. the stage of cauliflower homogenization, obtaining an extract with acetone seems to be more acceptable for the Allium test conditions.

1. Introduction

Focus on quality and safety of foods is currently increases, including the approaches to healthy eating and minimizing the risks of diseases associated with the diet of various populations. Particular attention is paid to food additives, as it is known that their use may cause toxic effects in the human body. Long-term consumption of certain additives with food is associated with the development of carcinogenic diseases, pathological changes in reproductive system and digestive tract [1,2,3]. However, these additives are often impossible to replace in the mass production of foods, where they are used as preservatives, sweeteners, colorants, emulsifiers, flavor enhancers and other components. Moreover, most products contain a mixture of additives, the toxic effect of which may have not only additive, but also a synergistic nature. It is important to note that the products of these additives' oxidation formed during the production and storage of foods are also chemically active compounds and show mutagenic activity [4].

All these problems require the development of a new methodology for testing products, which will lead to further revision of the toxicological hazard thresholds. Thus, it is proposed to concentrate the main efforts on studying the mechanisms of toxicant effects at the cellular and molecular level using modern methods of systems biology and bioinformatics, which allow predicting toxicity at the whole-body level. At the same time, the problem is being discussed of refusing animal experiments when possible [5]. Therefore, the use of vegetable test systems may be preferable in solving these scientific problems. We believe that the Allium test, in which onion roots are used as a test object, meets the specified conditions in the best way. This test is highly sensitive both to macro indicators (delayed root growth) and micro indicators (cytotoxicity, genotoxicity, level of oxidative activity biomarkers, etc.) and is especially suitable for the analysis of complex mixtures of chemical compounds. Currently, the Allium test is widely and successfully used to study the toxic, cytotoxic and genotoxic effects of food additives [6]. Several papers have also been published, in which onion roots were placed directly in food products such as drinks, juices, and milk [7,8,9]. However, these experiments are not so unambiguous, because high concentration of carbohydrates in drinks and juices, and the presence of emulsion in milk are undesirable factors in the Allium test. It seems more appropriate to test extracts of food products that are acceptable for the Allium test, which allows simultaneous and comprehensive assessment of various food additives and their mixtures. Such tests are especially relevant when developing new types of food products based on original recipes or technological methods of processing and storage. In the literature, we didn't find the data on extracts of food products to assess their toxicity in the Allium test, apart from the only work to detect pesticides in unwashed vegetables and grapes [10]. Therefore, the aim of the work was to develop methods for extracting food preservatives from preserved pureed vegetables, which are acceptable for further bioassay of these extracts in the Allium test.

2. Materials and methods

Benzoic acid (SIGMA-ALDRICH, lot.MKCG6487, Germany), sorbic acid (SIGMA-ALDRICH, lot.SLBW6722, Germany), and pureed cauliflower for infant nutrition purchased from a retail network were used as the test objects.

Preservatives (benzoic and sorbic acids) were added by weight to pureed vegetables individually and in a mixture, with a final mass fraction of 100 mg/kg.

3.1. Preparing the benzoic and sorbic acid stock solutions at a concentration of 1000 mg/dm^3

In a 100 cm^3 volumetric flask, the corresponding acids were weighed in amount of 0.1 g within the accuracy of 0.001 g, separately and together. 50 cm^3 of water was added to the mixture of acids followed by dissolving in a water bath at a temperature of 70 ± 5 °C for 30 minutes. The resulting solution was cooled to a temperature of 20 ± 5 °C and diluted with water to the volume of 100 cm^3.

FOR CITATION: **Samoilov A. V., Suraeva N. M., Koptsev S. V., Glazkov S. V.** Developing the methods of food preservatives extraction from complex matrices for bioassay purpose. *Food systems.* 2019; 2(4): 31–33. DOI: 10.21323/2618–9771–2019–2–4–31–33

3.2. Preparing a mixture of pureed vegetables with the preservatives

90 g of pureed cauliflower was placed in three glasses. 10 cm^3 of the sorbic acid stock solution (prepared according to Section 3.1) was added to the first glass to obtain a final concentration of 100 mg/kg in pureed vegetables. 10 cm^3 of the benzoic acid stock solution (prepared according to Section 3.1) was added to the second glass to obtain a final concentration of 100 mg/kg in pureed vegetables. 10 cm^3 of the benzoic and sorbic acid mixture stock solution (prepared according to Section 3.1) was added to the third glass to obtain a final concentration of 100 mg/kg in pureed vegetables. The resulting mixtures were thoroughly mixed on PE-6500 laboratory shaker (Acroshim, Russia) for 20 min at room temperature.

3.3. Extracting the preservatives from pureed vegetables

Mixtures of pureed vegetables with the preservatives (prepared according to Section 3.2) of 10 g each were transferred to 100 cm^3 volumetric flasks within the accuracy of 0.001 g. 40 cm^3 of acetone (EKOS-1, Russia) were added, mixed and placed in an ultrasonic bath (UNI0TRA UM-4, Poland) for 15 minutes at room temperature. Then it was diluted with acetone to obtain 100 cm^3, mixed and stored for 12 hours at 5 °C. After 12 hours, acetone mixtures were removed from the refrigerator, kept at room temperature for about 1 hour, and then filtered through a filter paper (Blue Ribbon). 2 cm^3 of each mixture filtrates were evaporated to dryness in Corning plastic tubes (Mexico) using Thermo Scientific evaporator (USA) in nitrogen stream for 40 min at 60 °C. Then, 2 cm^3 of HPLC water (Fisher Chemical, lot. 181206, Belgium) were added to the evaporated residue in the same tube and mixed with vortex (IKA vortex genius 3, Germany) for 1 min.

Similar extraction procedures were carried out under the same conditions, but instead of acetone, distilled water was used in the same amounts, excluding the evaporation and reconstruction procedures.

3.4. Determining the preservatives concentration in the extracts

The mass fraction of benzoic and sorbic acids was determined by high performance liquid chromatography (HPLC) method on Shimadzu Prominence LC-20 liquid chromatograph (Japan) in the ultraviolet range at a wavelength of 235 nm (benzoic acid) and 285 nm (sorbic acid), respectively.

A mixture of acetate buffer solution, pH 4.5 (Acros Organics, Belgium) and methanol (Merck Millipore, Germany) was used as the mobile phase. The components were separated using Hypersil BDS-C18 column (Thermo Scientific, USA), 150 × 4.6 mm, in isocratic mode, with a volume ratio of 75:25, while the eluent velocity was 1 cm^3 per minute.

The degree of extraction was estimated according to the concentration of the extracted preservatives.

3. Results and discussion

Preserved pureed cauliflower for infant food as a model matrix of complex composition was chosen for studies of the preservatives toxic effects in the Allium test. Extracts of such products are most likely do not contain any natural and synthetic toxicants, and therefore, the most accurate assessment of the toxic effects of food additives and their mixtures in this vegetable matrix is possible. According to the literature, in the Allium test, onion roots are sprouted, as a rule, in distilled or drinking water, in which the test compounds were dissolved; and the pH of the solution should be not lower than 3.5 and not higher than 11.0 [11, 12]. Stock solutions of benzoic and sorbic acids were within the indicated range. Therefore, in the beginning, the simplest extraction option was studied using distilled water. According to Table 1, the average degree of benzoic acid extraction from pureed vegetables was high and amounted to 82.6 %.

Table 1

Benzoic acid extraction results after aqueous extraction

The degree of benzoic acid extraction W_i, %	Average value, mg/kg	Standard deviation S, mg/kg	Recovery, % of the initial additive
78.92			
85.00	82.60	3.23	82.6
83.87			

However, despite the high degree of benzoic acid extraction, the aqueous extract from pureed vegetables did not meet the above Allium test parameters, due to the fact that it was a suspension. In bioassay, suspended particles could precipitate on onion roots and have a negative effect on their growth. We believe that the reason for such stable aqueous suspensions is homogenization of raw materials used in the manufacture of this type of pureed vegetables. All attempts to eliminate suspension and obtain a true solution, i.e. multiple dilution with water (4–8 times), centrifugation and filtering through filter paper were unsuccessful. On the other hand, it would be possible to dry pureed vegetables, and then obtain an aqueous extract of the dry residue, but this procedure is long and laborious and it is necessary to apply high heating temperatures, which may lead to the formation of additional toxicants in the extract. However, this extraction protocol can be used, for example, when testing tomato paste. It is known that in the production of tomato-containing products, other technological methods are usually used to achieve homogeneity, i.e. filtration and evaporation. In this case, it is most likely that the use of an aqueous extract will be more appropriate.

In order to eliminate the above disadvantages of pureed vegetables aqueous extraction, acetone was used as an extractant in the following experiments. Benzoic and sorbic acids are known to dissolve well in this solvent and its subsequent evaporation occurs under relatively mild temperature conditions (60 °C). As shown in Tables 2 and 3, the percentage of extraction for benzoic acid was only 6 % lower than in aqueous solution. Whereas, the average degree of sorbic acid extraction was even higher than for benzoic acid and amounted to 79.0 %. We believe that losses during extraction with acetone were associated with insufficient solubility of benzoic and sorbic acids as low-polar compounds (pKa 4.20 and 4.76, respectively). Therefore, it may be necessary to further dissolve the dry extract in water at a temperature of 70 °C. Based on the above, in the case of these preservatives, when analyzing the toxic effects of extracts in the Allium test, the correction factor of 0.8 must be considered. As far as we know, this is the first study on preparing the food extracts for bioassay, in which the effectiveness of the food additives extraction was studied. The quantification of extracted toxicants may be critical in understanding the mechanisms of their biological effects.

Table 2

Benzoic acid extraction results after extraction with acetone

The degree of benzoic acid extraction W_i, %	Average value, mg/kg	Standard deviation S, mg/kg	Recovery, % of the initial additive
67.79			
79.92			
73.92			
79.76	76.31	4.43	76.3 %
77.61			
79.76			
75.47			

Table 3

Sorbic acid extraction results after extraction with acetone

The degree of sorbic acid extraction W_i, %	Average value, mg/kg	Standard deviation S, mg/kg	Recovery, % of the initial additive
77.76			
72.70			
73.77			
71.64	79.0	6.57	79.0%
85.00			
86.48			
85.65			

To assess the interactions of preservatives and the specificity of their detection in pureed vegetables during extraction, experiments were carried out with their mixture in a concentration of 100 mg/kg of product. As shown in Table 4, the degree of extraction with acetone did not change significantly; the percentage of benzoic and sorbic acids extraction remained within the same range. It should be noted that when both preservatives present in the extract, evaluating the results in the Allium test require the correction coefficient to be increased twofold, as not only an additive, but also a synergistic effect is possible or other, more complex interactions.

Table 4

Preservatives mixture extraction results after extraction with acetone

Preservative	The degree of extraction W_i, %	Average value, mg/kg	Standard deviation S, mg/kg	Recovery, % of the initial additive
Benzoic acid	79.76	80.53	0.66	80.5
	80.91			
	80.91			
Sorbic acid	78.76	78.53	0.54	78.7
	77.92			
	78.92			

4. Conclusion

As a result of the research, two methods for obtaining extracts from preserved pureed vegetables acceptable for further bioassay in the Allium test were first studied. For the first time, studies have been conducted to evaluate the effectiveness of extraction for food preservatives added to the original product. The degree of extraction of these additives, benzoic and sorbic acids, was high and did not differ between both methods. However, in relation to physical and chemical characteristics, only the acetone extract from this specialized infant food met the requirements of the Allium test. We believe that the aqueous extract may also be successfully used in this test for other vegetable products that are not homogenized during the production.

REFERENCES

1. Olusegun, E.T., Olajire, A.A. (2015). Toxicity of food colours and additives: A review. *African Journal of Pharmacy and Pharmacology,* 9(36), 900–914. DOI: 10.5897/AJPP2015.4385
2. Bezerra, M. de S., Malaquias, G.de S., Castro e Sousa, J.M., Peron, A.P. (2016). Cytotoxic and genotoxic potential of powdered juices. *Food Science and Technology*, 36(1), 49–55. DOI: 10.1590/1678–457X.0006
3. Yu, B., He, J., Yu, J., Zheng, P., Yuan, Z., Chen, D., Xiangbing Mao, X. (2016). Excess of dietary benzoic acid supplementation leads to growth retardation, hematological abnormality and organ injury of piglets. *Livestock Science*, 190, 94–103. DOI: 10.1016/j.livsci.2016.06.010
4. Kitano, K., Fukukawa, T., Ohtsuji, Y., Masuda, T., Yamaguchi, H. (2002). Mutagenicity and DNA-damaging activity caused by decomposed products of potassium sorbate reacting with ascorbic acid in the presence of Fe salt. *Food and Chemical Toxicology,* 40(11), 1589–1594. DOI: 10.1016/s0278–6915(02)00119–9
5. Blaauboer, B.J., Boobis, A.R., Bradford, B., Cockburn, A., Constable, A., Daneshian, M., Edwards, G., Garthoff, J.A., Jeffery, B., Krul, C., Schuermans, J. (2016). Considering new methodologies in strategies for safety assessment of foods and food ingredients. *Food and Chemical Toxicology*, 91, 19–35. DOI: 10.1016/j.fct.2016.02.019
6. Samoilov, A.V., Suraeva, N.M., Zaitseva, M.V., Kurbanova, M.N., Stolbova, V.V. (2019). Comparative assessment of artificial sweeteners toxicity via express biotest, *Health Risk Analysis*, 2, 83–90. DOI: 10.21668/health.risk/2019.2.09.eng
7. Farhan, E.M., Tawfiq, R.F. (2016). Detection of Cytotoxicity and Carcinogenicity of Soft Drink «Cola» on Allium Cepa Root Cells. *International Journal of Advances in Agricultural and Environmental Engineering,* 3(1), 92–95. DOI: 10.15242/ijaaee.er01160020
8. Carvalho, B. de L., Sales, I. M. S., Peron A. P. (2017). Cytotoxic, genotoxic and mutagenic potential of UHT whole milk. *Food Science and Technology,* 37(2), 275–279. DOI: 10.1590/1678–457x.21916
9. Silva, E.L., Sousa e Sales, I.M., Silva dos Santos, F. K. Peron, A. P. (2017). Processed fruit juice ready to drink: screening acute toxicity at the cellular level. *Acta Scientiarum. Biological Sciences,* 39(2), 195–200. DOI: 10.4025/actascibiolsci.v39i2.33699
10. Feretti D., Zerbini I., Zani C., Ceretti E., Moretti, M., Monarca S. (2007). Allium cepa chromosome aberration and micronucleus tests applied to study genotoxicity of pesticide-treated vegetable extracts. *Food Additives and Contaminants*, 24(6), 561–572. DOI:10.1080/02652030601113602
11. Fiskesjo G. (1985). the Allium test as a standard in environmental monitoring. *Hereditas*, 102(1), 99–112. DOI: 10.1111/j.1601–5223.1985.tb00471.x
12. Kurbanova, M. N., Suraeva, N.M., Rachkova, V.P., Samoylov, A.V. (2018). Comparative study of indicators of toxic activity in the Allium-test. *Agrarian Bulletin of the Urals,* 4(171), 20–25. (in Russian)

AUTHOR INFORMATION

Artem V. Samoilov — candidate of biological sciences, chief of laboratory, Laboratory of quality and safety of foodstuffs, All-Russian Scientific Research Institute of Technology of Preservation — Branch of the V. M. Gorbatov Federal Research Center for Food Systems of RAS, 142703, Moscow region, Vidnoye, School str, 78, Tel.: +7–495–541–87–00, e-mail: molgen@vniitek.ru
*corresponding author

Natalia M. Suraeva — doctor of biological sciences, chief researcher, Laboratory of quality and safety of foodstuffs, All-Russian Scientific Research Institute of Technology of Preservation — Branch of the V. M. Gorbatov Federal Research Center for Food Systems of RAS, 142703, Moscow region, Vidnoye, School str, 78, Tel.: +7–495–668–70–37, e-mail: nsuraeva@yandex.ru

Sergey V. Koptsev — researcher, Laboratory of quality and safety of foodstuffs, All-Russian Scientific Research Institute of Technology of Preservation — Branch of the V. M. Gorbatov Federal Research Center for Food Systems of RAS, 142703, Moscow region, Vidnoye, School str, 78, Tel.: +7–495–668–70–37, e-mail: koptsev134@mail.ru

Sergey V. Glazkov — leading researcher, Laboratory of quality and safety of foodstuffs, All-Russian Scientific Research Institute of Technology of Preservation — Branch of the V. M. Gorbatov Federal Research Center for Food Systems of RAS, 142703, Moscow region, Vidnoye, School str, 78, Tel.: +7–495–668–70–37, e-mail: s.glazkov@outlook.com

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

The authors declare no conflict of interest

Received 18.10.2019 Accepted in revised 20.11.2019 Accepted for publication 05.12.2019