HYDROXYMETHYLFURFURAL IN CANNED FRUIT

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Abstract
A standard method for the quantitative determination of 5-hydroxymethylfurfural in canned fruit products using reversed-phase high-performance liquid chromatography and spectrophotometric detection in the ultraviolet region of the spectrum at a wavelength of 284 nm has been verified. The results of calculations of acceptance criteria during verification (repeatability and intermediate precision indices) showed that the considered method for determining the concentration of HMF in canned fruit described in GOST 31644-2012 is reproducible in a chromatographic research laboratory, and the obtained results can be considered reliable. It was shown that from 17.02 to 23.56 mg/dm^3 of HMF were detected in canned apples (puree and jam), which indicates the advisability of industrial control of the conditions of processing and storage of such products.

Keywords: canned fruit, hydroxymethylfurfural, reversed-phase high-performance liquid chromatography, measurement method verification

Introduction. The main criteria for the consumer value of processed fruit and vegetable products are their closeness to the original raw materials in terms of organoleptic properties and nutritional value, as well as the absence of foreign substances. It is known that the process of preserving fruits and vegetables, including heat treatment, leads to a change in the chemical composition of the original raw materials due to the resulting chemical reactions, which are the cause of a decrease in the content of biologically active substances, as well as the accumulation of compounds that are absent in the original raw materials and negatively affect the nutritional value of the final products. One of these compounds is hydroxymethylfurfural (5-hydroxymethylfurfural, HMF) (Figure 1), the content of which the International Association of Juice and Nectar Producers (AIJN) proposes to use as a criterion for the degree of impact of heat treatment, duration and storage conditions on the quality of fruit and vegetable juices and nectars. [1–5].

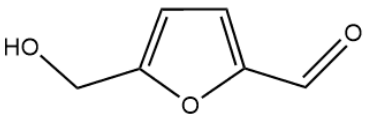

Fig. 1. Chemical structure of 5-hydroxymethylfurfural (HMF)

This substance is found in such food products as canned fruits and vegetables, wine products, soft drinks, roasted coffee, honey, bakery products. HMF is not found in plant materials and is practically absent in freshly prepared products. Its presence in food products is due to chemical transformations of carbohydrates, in particular, hexoses, occurring during the manufacturing and long-term storage of products [1, 2].

There are two known types of chemical transformations of hexoses leading to the formation of 5-hydroxymethylfurfural [1]. One of them is the so-called Maillard reaction, which is a complex multi-stage and branched process of interaction of carbohydrates with amino acids, peptides and proteins. The speed and depth of this interaction depends on the composition of the reacting products, the ratio of individual components, the pH of the environment, temperature and humidity. During the Maillard reaction, dark-colored products are formed – melanoidins. In this case, HMF is only a by-product of the reaction, which, along with other formed carbonyl compounds (furfural, acetaldehyde, isovaleric aldehyde, diacetyl, etc.), takes part in the formation of the aroma, color and taste of the finished product. This reaction often occurs during bread baking, drying fruits and vegetables, obtaining dry milk, evaporating sugar syrup, and during malt production.

Another way of forming HMF in food products is the reaction of dehydration of hexoses, which occurs under the catalytic action of acids. Heating causes dehydration of glucose, i.e. splitting off one or two water molecules from it. In this case, anhydrides are formed, reactive compounds that can combine either with each other or with an unchanged sucrose molecule and form so-called condensation (reversion) products. With prolonged heat exposure, a third water molecule is split off and hydroxymethylfurfural is formed, which, with further heating, can decompose with the destruction of the carbohydrate skeleton and the formation of formic and levulinic acid or form colored compounds.

The speed of these two reactions and, accordingly, the intensity of accumulation of HMF in the product largely depends on temperature. Therefore, depending on the path of formation of HMF, its content reflects the depth of chemical transformations of carbohydrates

or carbohydrates and protein substances during production, long-term storage or as a result of high-temperature processing of juice and confectionery products, honey and other sugar-containing products. A reliable correlation has also been established between the accumulation of HMF, color change and decrease in the content of bioflavonoids, which determine the biological value of fruit juices and purees.

Thus, the presence of hydroxymethylfurfural in food products is undesirable for the following reasons: furan derivatives are poisons, large doses of them cause convulsions and paralysis, small doses depress the nervous system. The human body cannot metabolize these compounds, which leads to their accumulation in the liver and disruption of biochemical processes in the body [2]. The toxic effect of HMF justifies the need to regulate its content in food products, especially in baby food products. In the EU countries, the content of HMF in juices and purees is limited to the maximum permissible value of 20 mg/l. In Belarus, the content of HMF is regulated by such documents as "Hygienic requirements for the quality and safety of food raw materials and food products" [6], technical regulations TR CU 021/2011 [7] and TR CU 023/2011 [8]. According to these documents, the content of HMF in juices and juice products, including raw materials for the production of baby food, should be no more than 10 mg/l (in juice products from citrus fruits) and 20 mg/l (in juice products from other fruits and vegetables), in honey and dietary supplements – no more than 25 mg/kg.

To control the content of hydroxymethylfurfural in food products, various methods are used, including colorimetric (Selivanov-Figuet reaction), spectrophotometric, chromatographic. The colorimetric method (Selivanov-Figuet reaction) is the simplest and most accessible, and is used for the qualitative determination of HMF in honey. The method is based on the formation of a cherry-red color product of the interaction of HMF with resorcinol in an acidic medium. The spectrophotometric method is used for the quantitative determination of HMF in juice products and consists of conducting a color reaction with barbituric acid with subsequent measurement of the optical density of the solution at a wavelength of 540 nm against a blank experiment. The sensitivity of the method is 2.0 mg/kg. The linearity range of the calibration dependence of the optical density on the HMF content is 2.0–30.0 mg/kg. However, this method is not suitable for determining HMF in citrus fruit processing products. The high-performance liquid chromatography (HPLC) method is used to measure the concentration of hydroxymethylfurfural in juices, juice products, beverages, dietary supplements, cognacs, wines, and honey. The HPLC method is based on the quantitative determination of HMF using reversed-phase high-performance liquid chromatography and spectrophotometric detection in the ultraviolet region of the spectrum at a wavelength of 284 nm. The range of concentrations determined is from 1.0 to 1000 mg/kg [1, 9, 10].

Thus, the problem of formation, accumulation and control of hydroxymethylfurfural content in food products is relevant and has scientific and practical significance. Also important is the selection and confirmation of the suitability of the appropriate method for measuring the concentration of HMF in a specific organic matrix. As practice has shown, the use of standard and certified measurement methods does not guarantee the reliability of the measurement results. Therefore, the testing laboratory must confirm its ability to correctly use standard methods before starting testing [11]. For this purpose, a measurement method verification procedure is carried out, i.e. providing objective evidence that the target measurement uncertainty can be achieved. Measurement methods with already established accuracy characteristics are subject to this procedure. In the experimental part of the verification procedure, the correctness of the measurement method and the processing of test data is checked, the quality indicators of the analysis results are assessed by comparing them with those specified in the method. Positive results of this stage indicate that the laboratory is able to obtain reliable analysis results using this method and can use it in the future [12–14].

Objective. Verification of a standard method for measuring the content of HMF in canned fruit using high-performance liquid chromatography.

Materials and methods. The objects of study in this work were samples of canned fruit selected from warehouses of finished products of processing enterprises of the Republic of Belarus. Information about the studied samples is given in Table 1.

Table 1

Brief information about the objects of study

Sample No.	Product name	Packaging type	Manufacturing date / Expiry date	Shelf life before testing, days
1	Multifruit juice with pulp	Plastic bottle, volume 1000 cm^3	27.02.2024 / 27.02.2025	17
2	Cherry juice	Plastic bottle, volume 1000 cm^3	02.03.2024 / 02.03.2025	13
3	Apple jam – semi-finished product, non-sterilized, grade 1	Polypropylene bucket with plastic handle, volume 1000 cm^3	01.02.2024 / 01.02.2026	28
4	Apple puree – semi-finished product, non-sterilized, grade 1	Polypropylene bucket with plastic handle, volume 1000 cm^3	25.01.2024 / 25.07.2025	33

The subject of the study was the content of hydroxymethylfurfural, which was determined according to the requirements of the standard GOST 31644-2012 [15], which establishes a method for the quantitative

determination of 5-hydroxymethylfurfural using reversed-phase high-performance liquid chromatography and spectrophotometric detection in the ultraviolet region of the spectrum at a wavelength of 284 nm. The stages of performing measurements with an indication of the necessary measuring and testing equipment, reagents, laboratory glassware and the required measurement conditions are presented in Figure 2.

1. Preparation for the determination

1) water for laboratory analysis of the 1st degree of purity - 840 cm3;
2) acetonitrile - 150 cm3;
3) acetic acid of special purity - 10 cm3;
4) unit for degassing the eluent;
5) measuring flask with a capacity of 1000 cm3;
6) membrane filter with a pore diameter of 0.45 μm.

1. Preparation of mobile phase for liquid chromatography
1.1. Mix water, acetonitrile and acetic acid in sequence.
1.2. Transfer the solution to the eluent container and mix.
1.3. Degas the resulting solution under vacuum (15 min) with simultaneous filtration.

Fig. 2. Scheme for measuring the concentration of HMF using the HPLC method according to [15] (start)

1) 5-HMF – 0.025 g;
2) acetonitrile – 25 cm^3;
3) analytical scales;
4) beaker with a capacity of 50 cm^3;
5) measuring flask with a capacity of 25 cm^3

2. Preparation of the basic standard solution of 5-HMF (c=1000 mg/dm^3)
2.1. Weigh a sample of 5-HMF.
2.2. Dissolve in acetonitrile, bring to the specified volume and mix thoroughly.

1) basic standard solution of 5-HMF – 200 μl;
2) acetonitrile – 50 cm3;
3) water for laboratory analysis;
4) spectrophotometer;
5) quartz cuvettes with an optical path length of 10 mm;
6) measuring flask with a capacity of 50 cm3;
7) pipette dispenser.

3. Preparation of a working standard solution of 5-HMF (c= 1–7 mg/dm^3)
3.1. Mix acetonitrile and an aliquot of the 5-HMF stock solution in sequence.
3.2. Dilute with acetonitrile to the specified volume and mix thoroughly.
3.3. Determine the absorption spectrum of the 5-HMF working solution relative to water.
3.4. Calculate the exact mass concentration of the 5-HMF working standard solution using the equation:

$$C_{working} = 1000 \cdot A \cdot M/\varepsilon \cdot L = A \cdot 7{,}487$$

3.5. Calculate the exact mass concentration of the basic standard solution of 5-HMF using the equation:

$$C_{main} = C_{working} \cdot r$$

1) main standard solution 5-HMF – 1.6 cm^3;
2) calibration solution No. 1 – 0.78 cm^3; 3.13 cm^3; 12.5 cm^3;
3) distilled water;
4) measuring flasks with a capacity of 50 cm^3;
5) pipette dispenser.

4. Preparation of calibration solutions of 5-HMF (C = 1.0–32.0 mg/dm^3)
4.1. Take an aliquot of the main standard solution of 5-HMF, bring to the specified volume with distilled water and mix thoroughly (calibration solution No. 1).
4.2. Take an aliquot of solution No. 1, bring to the specified volume with distilled water and mix thoroughly (calibration solutions No. 2–No. 4)

2. Taking measurements

1) calibration solutions No. 1–No. 4;
2) mobile phase (item 1);
3) chromatographic column;
4) chromatograph.

5. Construction of calibration dependence
5.1. Conduct a chromatographic analysis of all calibration solutions and record the peak areas of 5-HMF.
5.2. Plot a calibration graph – dependence of the peak area on the mass concentration (mass fraction) of 5-HMF in the calibration solution.

1) sample; 2) distilled water; 3) analytical scales; 4) pipette dispenser; 5) glass rod; 6) measuring flask; 7) beaker; 8) medical syringe; 9) vial.	**6. Preparing samples for measurement** 6.1. Weigh the sample or take an aliquot of the sample (mix first if necessary). 6.2. Dilute with distilled water to the specified volume and mix thoroughly. 6.3. Filter into a vial.
1) sample; 2) mobile phase (p.1); 3) chromatographic column. 4) chromatograph.	**7. Sample analysis** 7.1. Conduct a chromatographic analysis of the samples and record the peak area of the 5-HMF. 7.2. Calculate the mass concentration or mass fraction of 5-HMF using calibration curves, taking into account the degree of sample dilution.

Fig. 2. Scheme for measuring the concentration of HMF using the HPLC method according to [15] (finish)

The measurements were carried out on a high-performance liquid chromatograph (chromatographic column "*Superspher® 100 RP-18*" (250×4.6)) of the "*Agilent 1200*" type with a spectrophotometric detector (working range of absorption wavelengths from 190 to 600 nm) and a hardware and software complex for collecting and processing the results. A *Cary 50 Scan* spectrophotometer was also used in the research. The tests were carried out by two operators in the chromatographic research laboratory under the following conditions: ambient air temperature – (25±5) °C; atmospheric pressure – (97±10) kPa; relative air humidity – (65±15) %; alternating current frequency – (50±5) Hz; network voltage – (220±10) V. Verification criteria were calculated using the formulas given in [16].

Results and discussion. The results of the chromatographic analysis of all calibration solutions for constructing the calibration graph are given in Table 2. An example of a chromatogram of one of the calibration solutions is shown in Figure 3.

Table 2

Data for constructing a calibration graph

Mass concentration 5-HMF, mg/dm^3	Peak area, mAU s		Average value of peak area, mAU s
	Parallel 1	Parallel 2	
0,851	28,708	28,756	28,732
3,415	119,260	118,610	118,935
13,64	485,920	485,770	485,845
27,28	954,650	953,490	954,070

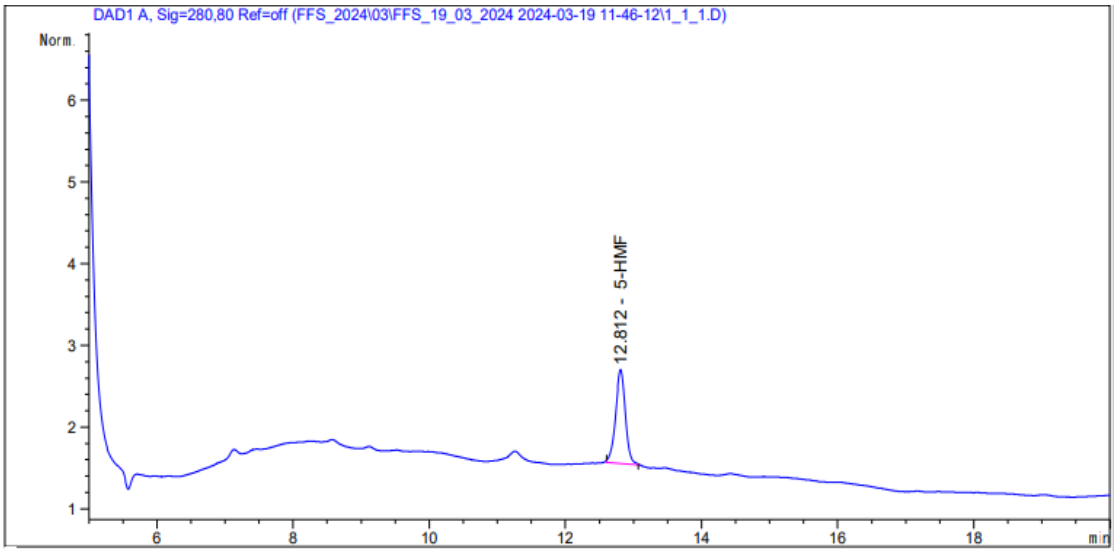

Fig. 3. Example of a chromatogram of a calibration solution with a mass concentration of 3.415 mg/dm3

To construct a calibration graph (Figure 4) and process the obtained data, the *Instrument 1 offline* program was used.

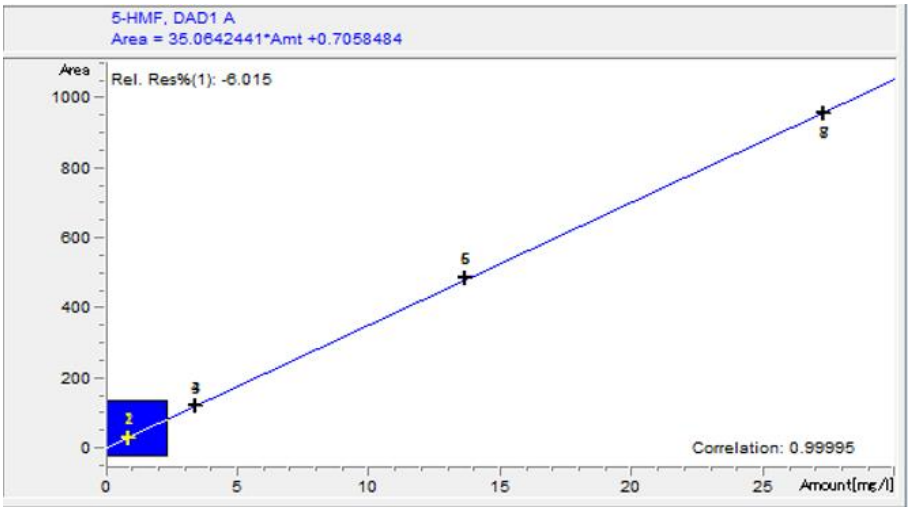

Fig. 4. Calibration graph

The results of the chromatographic analysis of the samples of canned products are presented in Table 3.

Table 3

Results of the chromatographic analysis of the samples of canned fruit

Date of test	Operator	Average values of HMF concentration ($\overline{c}$, mg/dm^3) of samples::			
		1	2	3	4
21.03.2024	1	3,063	0,405	23,263	16,927
	2	3,064	0,396	23,263	16,929
22.03.2024	1	3,051	0,423	23,748	17,054
	2	3,043	0,417	23,758	17,061
25.03.2024	1	3,088	0,393	23,473	17,095
	2	3,099	0,389	23,477	17,087
26.03.2024	1	3,290	0,347	23,648	16,861
	2	3,284	0,375	23,678	16,864
27.03.2024	1	3,302	0,438	23,642	17,159
	2	3,299	0,442	23,627	17,166
Average value, mg/dm^3		3,16	0,40	23,56	17,02
MPL, mg/l		20,0			

An analysis of the measurement results presented in Table 4 allows us to draw the following conclusions:

– the highest content of HMF was found in the canned apple samples (No. 3 and No. 4), which may be due to several reasons: the reaction between fructose (5.9 g in 100 g of apples) and amino acids of apple raw materials at high temperatures and low pH, as well as the process of caramelization of sugar added during jam cooking;

– the low concentration of HMF in fruit juice products (samples No. 1 and No. 2) may be due to the use of gentle heat treatment, as well as a lower sugar content in the product;

– the repeatability conditions [16] were met, since the same method and identical samples of the test object were used, the measurements were carried out by all participating operators in the same laboratory and on the same equipment in a short period of time.

The results of calculations of the acceptance criteria during verification (repeatability and intermediate precision indices) showed that the considered method for determining the concentration of HMF in canned fruit using reversed-phase high-performance liquid chromatography and spectrophotometric detection in the ultraviolet region of the spectrum at a wavelength of 284 nm, described in [15], is reproducible in a chromatographic research laboratory, and the results obtained can be considered reliable.

Conclusions. Thus, the results of the conducted studies and corresponding calculations allow us to draw a conclusion about the feasibility of production control of canned apple products in terms of the conditions of their processing and storage in order to minimize the risk of HMF formation. It should also be noted that when choosing a method for measuring the content of hydroxymethylfurfural in products, it is necessary to determine the repeatability criterion, which allows us to assess the degree of variability of the measurement results or experiment when they are repeated. Knowing the repeatability criterion, we can determine the per-

missible measurement error, which is important for ensuring the accuracy, reliability and quality of the measurement results.

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