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# STEP-BY-STEP CONTROL OF THE TECHNOLOGICAL PROCESSES IN THE PRODUCTION OF KAPPASPIN SUBSTANCE

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**Abstract**

This article presents the results of experiments conducted to determine the loss of the main product and yield efficiency during the technological stages of producing the Kappaspin substance from the buds and unripe fruits of the *Capparis spinosa* L. plant. It also describes the method for determining the residual amount of the organic solvent ethanol in the cappaspin substance. The results showed that the loss of Kappaspin substance, based on the plant raw material, accounted for 25%, while unaccounted losses were 5%. The yield efficiency of Kappaspin substance was found to be 70%. Furthermore, it was determined that the allowed maximum concentration of ethanol, an organic solvent in the Kappaspin substance, should not exceed 0.5%, or 5000 ppm.

**Keywords:** *Capparis spinosa L. substance, Kappaspin, analytical method, technological properties*

## Introduction

The literature mentions that the unripe fruits and flowers of *Capparis spinosa* L. are rich in vitamins. Its fruit contains mainly vitamins K and B, potassium, calcium, iron, magnesium, and many other elements, displaying various biological activities (Tazhibaev G. G., Inagamov S. Ya., 2023; Farzad Kianersi, Mohammad Reza Abdollahi, Asghar Mirzaie-asl, Dara Dastan & Faiza Rasheed 2020; Sambasivam Manikandaselvi, Pemaiah Brindha, Vellingiri Vadivel. 2018; Murodjon Isagaliev, Evgeny Abakumov, Avazbek Turdaliev, Muzaffar Obidov, Mavlonjon Khaydarov, Khusnida Abdukhakimova, Tokhirjon Shermatov, and Iskandar Musaev. 2022). The above-ground part of *Capparis spinosa* L. contains 0.32% rutin, quercetin, up to 150 mg/% vitamin C, stachydrin, thioglucoside, saponins, coloring substanc-

es, while the fruit contains up to 36% sugars, 25–25.6 mg/% vitamin C, 1.46% flavonoids, and thioglucoside. The seed contains 25–36% oil, the root contains 1.2% alkaloids (stachydrin), 0.44% flavonoids, 4.5% sugars, coumarins, and other substances. The buds (flowers) of *Capparis spinosa* L. contain 25% protein, 3% fat, 5% rutin, 150 mg/% vitamin C, and others. Avicenna (Ibn Sina) used *Capparis spinosa* L. to treat respiratory diseases, gastrointestinal disorders, and as a pain reliever, wound healer, and emetic. The primary usable parts of the plant are the flower buds and unripe fruits. Additionally, in the traditional medicine of China, Iran, Morocco, Pakistan, Egypt, and Arab countries, various parts of the plant (roots, stems, and leaves) are widely used (Tlili N., Elfalleh W., Saadaoui E., Khaldi A., Triki, S., Nasri N., 2011).

Mishra P. R. and (Mishra P. R., Panda P. K., Chowdary K. A. and Panigrahi S., 2012) his co-authors studied the effects of the fruit extract of *Capparis spinosa* L. on diabetic conditions in rats. The results showed a decrease in plasma glucose levels, an increase in insulin levels, and restoration of glycogen composition and enzyme activity related to carbohydrate metabolism. Histopathological examination of the pancreas revealed the enlargement of Langerhans islets and a reduction in neutral fats.

At the Institute of Plant Chemistry, scientific research was conducted on the development of a technology for producing the Kappaspin substance, composed of polysaccharide aggregates, from the buds and unripe fruits of *Capparis spinosa* L. (Botirov R. A., Saidova G. E., Mutalova D. K., Sanoev A. I., Sadikov A. Z., Sagdullaev Sh. Sh., 2022). As a result of the studies, a production technology for the Kappaspin substance was developed, and the polysaccharide content, monosaccharide residues, and pharmacotoxicological properties of the obtained samples were determined (Botirov, Ruzali A., Saidova, Gavkhar E., Mutalova, Dilnoza K., Sadikov, Alimdjan Z., and Sagdullaev, Shamansur Sh., 2024; Zhauynbaeva K. S., Botirov R. A., Sadikov A. Z., Sagdullaev Sh. Sh., 2022; Mirzaev, Ruzimov E. M., Botirov R. A., Saidova G. E., Sadikov A. Z. Sagdullaev Sh.Sh., 2023; Bekmurzayeva Nurjamal Baxtiyarovna, Botirov Ruzali Anvarovich, Azamatov Azizbek Azamatovich, Tursunkhodzhaeva Firuza Muratovna, Aytbayeva Aygul Baxtiyorovna, Sadykov Alimdjan Zairovich, Sagdullayev Shamansur Shaxsaidovich. 2024; Bekmurzayeva N. B., Botirov R. A., Azamatov A. A., Tursunkhodzhaeva F. T. Sadykov A. Z., Sagdullayev Sh. Sh., Sultanova R. K., 2024). Continuing our research, we aimed to determine the loss of polysaccharides and yield efficiency during the technological processes of Kappaspin substance production. For this, we determined the quantitative proportion of polysaccharides in the buds and unripe fruits of *Capparis spinosa* L. using the following method.

## Materials and methods

An analytical sample of the raw material was taken and ground until it passed through a sieve with 2 mm holes. A 10 g (accurately measured) portion of the ground, sieved sample was placed into a 250 ml volumetric flask with a round bottom, to which 200 ml of water was added. The flask was connected to a condenser and heated on an electric stove for 30 minutes while stirring. The extraction was repeated twice: first with 200 ml of water, then with 100 ml of water. The aqueous extracts were combined and centrifuged for 10 minutes at 5000 rpm. Next, a 500 ml volumetric flask was taken, and a glass funnel with a 55 mm diameter was placed on it, covered with a 5-layer gauze soaked in water. The extract from the centrifuge was passed through the gauze, washed with water, and the volume was adjusted to the mark with water (solution A).

From solution A, 25 ml was taken, placed in a centrifuge tube, and 75 ml of 95% ethanol was added, mixed well, heated in a water bath at 30 °C for 5 minutes, and left for 1 hour. After the designated time, the mixture was centrifuged for 30 minutes at 5000 rpm. The liquid was separated, and the residue was dried at 100–105 °C until a constant weight was achieved. A 16 mm diameter glass filter with holes was used under vacuum pressure (13–16 kPa) to filter the residue. The remaining sediment was filtered again, washed with 15 ml of 95% ethanol-water mixture (3:1), followed by 10 ml acetone and 10 ml ethyl acetate. The filter containing the sediment was dried first in the open air and then at 100–105°C until a constant weight was achieved.

The content of polysaccharides in the plant raw material (X) was calculated based on the absolute dry weight using the following formula:

$$X = \frac{(m_2 - m_1) \cdot 500 \cdot 100 \cdot 100}{m \cdot 25 \cdot (100 - W)}$$

Here, $m_1$ – is the mass of the filter in grams;

$m_2$ – is the mass of the filter along with the sediment in grams;

$m$ – is the mass of the raw material in grams;

$W$ – is the percentage of mass loss during the drying of the raw material.

The content of polysaccharides in the samples of *Capparis spinosa* L. buds varies between 9.5% and 15.5%, depending on the vegetative phase and the growth location.

In the immature fruits of the plant, this indicator was 4.5–7.8%. Each analysis was repeated at least three times for each sample.

The final technological stage in the production of the substance obtained from the buds and immature fruits of *Capparis spinosa* L. involves the precipitation of polysaccharides in ethyl alcohol and their washing, which necessitated the control of the residual ethyl alcohol content in the substance. To determine the residual ethyl alcohol, gas-liquid chromatography was used. Chromatograms were obtained on a gas-liquid chromatograph under the following conditions, each sample being taken at least 5 times:

– Flame-ionization detector;
– 3.0×2500 mm glass column;
– Sorbent with particle size of 0.16–0.20 mm, N-AW-DMCS;
– 5% SE-52 liquid phase;
– Evaporator temperature 150 °C;
– Column temperature 40 °C;
– Carrier gas (nitrogen) flow rate 30 ml/min.

For obtaining chromatograms, a sample of 0.5 g (accurate weight) of the polysaccharide substance was taken and placed in a 25 ml volumetric flask. 5 ml of water for injection was added to the flask, and the sample was dissolved. The solution was then made up to the mark with the same solvent. The solution was filtered through a glass filter with pores of 0.45 μm, and 1.0 μl of the filtered solution was injected into the chromatograph's injector. Also, a working standard sample of ethyl alcohol was prepared. 25 ml of water, cooled to 10 °C, was added to a 100 ml volumetric flask, followed by 0.2 g (accurate weight) of ethyl alcohol. The mixture was stirred. Then, more water was added to bring it to volume, and the mixture was stirred (Solution A). 50 mL of Solution A was transferred to a 100 ml volumetric flask, and water was added to bring it to volume and stirred (Solution B). 5 ml of Solution B was transferred to a 100 mL volumetric flask, and water was added to bring it to volume and stirred (Solution C). The solution should be used within 30 minutes of preparation. Checking the suitability of the chromatographic system. The ethyl alcohol standard sample (1.0 μL) is injected five times. The chromatographic system is considered suitable if the separation factor between the two components is at least 1.0 and the relative standard deviation of the repeatability is no more than 15%. Identification of ethyl alcohol is performed by comparing the retention times of the peaks of the sample being analyzed and the ethyl alcohol standard. Method. 1.0 μL of the sample to be analyzed and the ethyl alcohol working standard are injected into the apparatus. Five chromatograms are taken, and the area of each peak is recorded. The residual ethyl alcohol content in the preparation (Q, %) is calculated using the following formula:

$$Q = \frac{S_i \cdot 25 \cdot M_m \cdot 100\%}{S_m \cdot M_i \cdot 100_1}$$

"Here; $S_i$ – the area of the peak of the sample being analyzed in the chromatogram, mm²;

25 – the volume of the sample being analyzed, ml;

$M_m$ – the amount of the ethyl alcohol working standard, g;

$S_m$ – the area of the peak of the ethyl alcohol standard in the chromatogram, mm²;

$M_i$ – the amount of the sample being analyzed, g;

100 – the volume of ethyl alcohol, ml.

The efficiency of this method is equal to 1.05. If the efficiency criterion (N) is within the range of 1 to 2, the system is considered efficient.

It was determined that the residual amount of ethyl alcohol in all the samples

analyzed was below the allowed upper limit (3550–4800 ppm).

## Results and Discussion

During the experiments, the total amount of water-soluble polysaccharides in the raw materials, alcoholic extracts, aqueous extracts, press cake, chloroform extract, aqueous residue, technical product, alcoholic residue, and final product was analyzed. The results of the yields and losses of the main product are presented in the following table (table 1).

**Table 1.** *Step-by-step control of technological processes (main yields and losses)*

| Objects being studied | Amount of Kappaspin substance, % | |
|---|---|---|
| | Relative to the mass of the raw material | Yield efficiency relative to the amount in the raw material |
| Raw material | 6 | 100 |
| Alcoholic extract | 0,3 | 5,0 |
| Aqueous extract | 5,4 | 90 |
| Plant residue (press cake) | 0,3 | 5,0 |
| Chloroform extract | 0,3 | 5,0 |
| Aqueous residue | 5,1 | 85 |
| Technical product | 4,5 | 75 |
| Alcoholic solution | 0,6 | 10 |
| Kappaspin substance | 4,2 | 70 |
| Unaccounted losse | 0,3 | 5,0 |

## Conclusions

As a result of the scientific research conducted, a step-by-step control method for the technological processes of *Capparis spinosa* L. plant's water-soluble polysaccharide yield in industrial production was developed for the Kappaspin substance. According to this method, the losses accounted for in the plant raw material were determined to be 25%, unaccounted losses were 5%, and the yield of the Kappaspin substance was 70%, as established through experiments. It was also determined that the maximum allowable concentration of the organic solvent, ethyl alcohol, in the Kappaspin substance is 0.5%, or 5000 ppm.

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