RESEARCH ARTICLE | DECEMBER 07 2023

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AIP Conf. Proc. 2889, 080015 (2023)
https://doi.org/10.1063/5.0172815

Development and research of a drink with antioxidant properties obtained from a combination of dried rose hips and a cheese-making by-product

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Abstract. The composition and characteristics of the by-products (whey) in the production of yellow cheese and cheese depend on the technology of their production and the quality of the milk used. The aim of the study is to obtain a beverage with antioxidant properties from whey, by direct extraction with dried rose hips (*Rosa canina* L.) With the best indicators of antioxidant activity and vitamin C content observed in the beverage (sample S1) , obtained at the following technological parameters: hydromodule 1:20, temperature 0-4 ºC; extraction duration 3 h. The beverages were analyzed at regular intervals over a 15-day storage period. After adapting and researching a computer-based method and tools for automated determination of a suitable hydromodule for obtaining a whey beverage, it was predicted that this is a 1:24 hydromodule, which is close to the experimental one. The method of direct extraction of dried fruits in whey can be used in the development of new functional drinks and successful utilization of by-products of the cheese industry.

INTRODUCTION

Whey is a liquid by-product of white brined cheese and yellow cheese production. The composition and characteristics of whey depend on both the technology of production of the cheese products and the quality of the milk used. Liquid whey consists of about 93-94% water and 6-7% dry matter. The nutritional and biological value of whey is determined by the protein substances, mineral salts, lipids, vitamins, organic acids, enzymes, carbohydrates, immune bodies and trace elements contained in it.

The content of protein substances in whey is relatively low (about 1%), but their biological value is high because they contain all the essential amino acids necessary for the human body. Whey proteins have a neutral pH, so they can be used in the production of functional drinks [1].

Whey-based beverage production began in the 1970s and a wide range of different whey beverages have been developed to date [2].

Annually, about 1.2 million tons of lactose and 200,000 tons of milk protein worldwide are discarded in whey production, of which only 60% is used for human and animal feed [3].

The utilization of whey in the form of concentrate or whey protein is observed in the production of various types of dairy products and bakery products [4].

Turning whey into a drink by adding fruit is one way to enrich it with biologically active substances. Various whey drinks have been developed with the addition of the tropical fruit guava, mango pulp, papaya and others. Another method for enriching whey with biologically active substances is the addition of extracts of various fruits [5, 6].

Rose hips (Rosa canina L.) are characterized by a high content of biologically active components such as: vitamins (C, B, P, PP, E, K), flavonoids, carotenes, carbohydrates (mono- and oligosaccharides), organic acids (wine, lemon), microelements, etc., which makes it a suitable raw material for obtaining enriched extracts [7].

No methods have been established for the direct extraction of fruit into whey, for the extraction of biologically active substances and for the production of an fortified beverage.

International Conference on Technics, Technologies, and Education
AIP Conf. Proc. 2889, 080015-1–080015-14; https://doi.org/10.1063/5.0172815
Published by AIP Publishing. 978-0-7354-4760-8/$30.00

The aim of the present study is to obtain a whey beverage with antioxidant properties by performing direct extraction with dried rose hips.

MATERIAL AND METHODS

Dried rose hips (*Rosa canina* L.) purchased from the commercial network were used. Prior to extraction, the fruits are washed, dried and ground to a size of 2.0-4.0 mm. The chemical parameters of the dried rose hips (*Rosa canina* L.) used are given in Table 1. The chemical composition of the analyzed fruits is comparable with the data from the literature. Rose hips are evaluated primarily by the content of ascorbic acid (vitamin C), tannins and pectin. Rose hips can accumulate up to 1000-4000 $mg.g^{-2}$ ascorbic acid and 4.5-6.8% tannins [8,9].

On the dried fruits of rose hips (*Rosa canina* L.) are determined: dry matter; titratable acidity; ascorbic acid, tannins (tannins), Table 1.

Dry matter, % – according to [10].

Titratable activity, % – according to [11].

Ascorbic acid, mg/100g – by extraction with water at room temperature and titration of the obtained filtrate with 0,001 N 2,6-dichlorophenolindophenol (Tillmans reagent) in acidic medium, mg%.

Tannins,% – by exhaustive extraction with hot water at reflux and titration of the obtained extract with 0,1n $KMnO_4$ at indigo carmine indicator.

Table 1. Physico-chemical characteristics of dried rose hips

Dry matter, %	Titratable Acidity, %	Ascorbic acid, mg/100g	Tanning substances, %
88.7±0,8	2.4±0.1	1840.9±3.6	4.5±0.2

The extraction was performed with liquid whey obtained from the production of cow's milk cheese from the company "BG Yogurt" Yambol, Bulgaria.

The chemical composition of whey is shown in Table 2.

Table 2. Chemical composition of whey

Product	Dry matter,%	Proteins,%	Lactose,%	Fat,%	Minerals,%
Cheese whey	6.7	0.7	4.9	0.4	0.7

To obtain a whey drink with antioxidant properties, a direct extraction of dried rose hips was carried out in it. To improve the taste of the beverage during extraction, a cinnamon stick of 0,1 g was added. The extraction was performed at the following technological parameters: hydromodules 1:20 (S1), 1:30 (S2), 1:40 (S3), 1:50 (S4) and 1:60 (S5); temperature 0-4°C; extraction duration 3h. The notations used are summarized in Table 3.

Table 3. Notations used

Sample	S0	S1	S2	S3	S4	S5
Hydromodule	Control	1:20	1:30	1:40	1:50	1:60

After extraction, the whey is filtered to separate the solids from the rose hips, then pasteurized at 60-65 °C for 30 minutes. The pasteurized whey is cooled and stored in refrigerated conditions at a temperature of 0-4 ºC. Samples of the obtained enriched whey are analyzed both on the day of their receipt and on the 5th, 10th and 14th day of their storage.

Table 4 describes the steps for preparing a whey drink with direct extraction of rose hips.

Table 4. Stages of obtaining whey-rosehip drink

Stage	Name	Description
A	Preparation and qualification of whey	Receipt and qualification
B	Extraction	Extraction with dried rose hips and cinnamon
C	Filtering	Separation of rosehip and cinnamon solids
D	Pasteurization	60-65 ºC for 30 min
E	Cooling	At 0-4 ºC
F	Storage	At 0-4 ºC

On the resulting whey drink are determined:

Active acidity, pH – potentiometrically, by pH meter (Model MS 2011, Microsyst, Plovdiv, Bulgaria), equipped with an electrode (pH electrode Sensorex, Garden Grove, CA, USA);

Proteins, % – Reference method [12];

Fat content (milk fat), % – according to the method of Gerber [13];

Lactose, % – [14];

Ash content, % – according to Kirdar et al. [15];

Dry matter, % – according to BNS [16];

Ascorbic acid, mg/100g – by the method described by Gjorgievski et al. [17]

Antioxidant activity, % – The test extract is mixed with freshly prepared DPPH solution (2,2-diphenyl-1-picrylhydrazyl). The reaction mixture was incubated in the dark for 15 minutes at 37 °C. The absorbance reduction was read spectrophotometrically at 517 nm. Spectrophotometric measurements were performed with a UV-VIS spectrophotometer HALO SB-10 (Dynamica Scientific Ltd.). The spectrophotometer has a measurement range of 250-1200nm;

Electrical conductivity (EC), μS – via Conductivity Meter AP-2 (HM Digital, Inc). The device is suitable for measuring the electrical conductivity of aqueous solutions of salts, acids and bases;

Total dissolved solids (TDS), ppm – using a TDS-3 measuring instrument (HM Digital, Inc.);

Oxidation-reduction potential (ORP), mV – by Measuring Instrument Model ORP-2069 (Shanghai Longway Optical Instruments Co., Ltd).

Color of the drink – spectrophotometrically according to the methodology presented in [18]. The absorbance values at 420, 520 and 620 nm were determined. They are converted to XYZ and then to Lab color components. From the color obtained in the Lab color model, the yellow index (YI) and the white index (WI) are determined.

$$YI = \frac{142{,}86b}{L}; \quad WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \tag{1}$$

Organoleptic analysis and sensory evaluation - Sensory evaluation was performed with 15 respondents from the academic staff of the Faculty of Engineering and Technology, Yambol, Bulgaria. The control and the resulting beverage were evaluated on a 9-point scale, according to Fernández-Vázquez et al. [19]: from 1 – does not correspond completely to 9 – has full compliance with the indicator.

A total of 29 features were selected to describe the whey drink obtained. The features are presented in Table 5. They include: physicochemical, optical, color and organoleptic characteristics of the product.

Table 5. Features describing the characteristics of whey-rosehip drink

Feature	Characteristic	Feature	Characteristic	Feature	Characteristic
F1	pH	F11	*AOA, %	F21	YI
F2	EC, μS	F12	*A_{420}, AU	F22	Appearance
F3	TDS, ppm	F13	*A_{520}, AU	F23	Color
F4	ORP, mV	F14	*A_{620}, AU	F24	Odor
F5	Protein, %	F15	L	F25	Taste
F6	Fat, %	F16	a	F26	Viscosity
F7	Lactose, %	F17	b	F27	Homogeneity
F8	Ash, %	F18	C	F28	Flavour
F9	Dry matter, %	F19	h	F29	OA
F10	*AA, mg/100g	F20	WI	-	-

*AA – Ascorbat Acid; AOA – antioxidant activity; A_{420}, A_{520}, A_{620} – Absorbance at 420, 520 and 620 nm.

For the selection of informative features the method "Correspondence analysis" was used [20]. The task in conformity analysis is similar to that in principal component analysis (PCA). Unlike PCA, which is applied to quantitative traits, compliance analysis is applied to qualitative traits. The values of the weights wi and wj by rows and columns of the data table are obtained from the vectors r and c:

$$w_i = \{r_i\}; \quad w_j = \{c_j\} \tag{2}$$

The coefficients of the regression model are determined. It is estimated by standard error (SE), p-value, Fisher's test (F).

A linear programming algorithm was used to determine the appropriate beverage hydromodule. This algorithm is implemented through the linprog function in Matlab.

The results of this analysis are visualized by composition-characteristic diagrams (simplex diagrams). They reflect the influence of the hydromodule on specific characteristics of the beverage [21].

The measurements were made with three repetitions. The analyzes are at a level of significance α=0,05.

RESULTS AND DISCUSSION

On the first day after preparation of the drink, active acidity (pH) electrical conductivity (EC), completely dissolved solids (TDS) and redox potential (ORP) were determined. The results of this analysis are presented in Table 6. The data in the table show that with increasing hydromodule, the electrical conductivity (EC) and TDS increase and the pH decreases compared to the control sample. This is probably due to the extracted substances and organic acids in the whey, whereby the redox potential is improved. Therefore, by the process of extraction of rose hips in whey, it is possible to improve the antioxidant properties of the resulting beverage. This is confirmed by data on the content of vitamin C and antioxidant activity in beverages, traced at the beginning and during the storage period. The results are shown in Fig.1 for vitamin C and Fig. 2 for antioxidants.

Table 6. Characteristics of whey and rosehip drink

Sample	pH	EC, µS	TDS, ppm	ORP, mV
S0	6.6±0.3	1731±62	306±34	272±21
S1	5.7±0.2	1720±54	342±22	-364±32
S2	5.8±0.1	1759±24	350±26	-157±12
S3	5.9±0.2	1759±16	362±11	-95±4
S4	5.9±0.3	1759±21	374±8	-32±6
S5	6.1±0.5	1705±43	358±31	112±16

The values have statistically significant difference at $p<0.05$. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined.

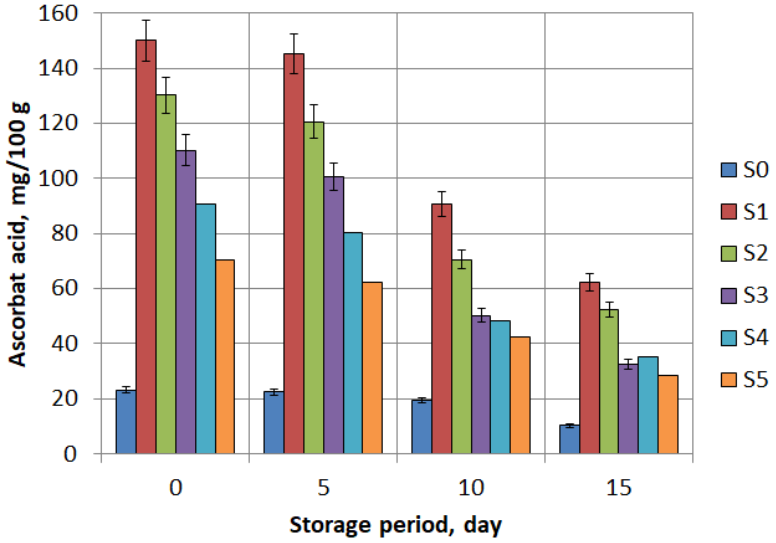


FIGURE 1. Content of vitamin C in the drink during storage.

The values have statistically significant difference at $p<0.05$. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined.

Higher values of vitamin C were observed in samples S1 (150.09 mg/100g). S2 (130.25 mg/100 g) and S3 (110.25 mg/100g). During storage of the beverage. the vitamin C content in the samples decreased. with a more significant decrease in sample S1 (62.2 mg/100g) on day 15 of storage. as shown in Figure 2. It was shown that the degradation of vitamin C during storage is affected by the action of certain mineral elements. metals. enzymes. ascorbate oxidase. phenolase. peroxidase) or oxygen from the air [22. 23].

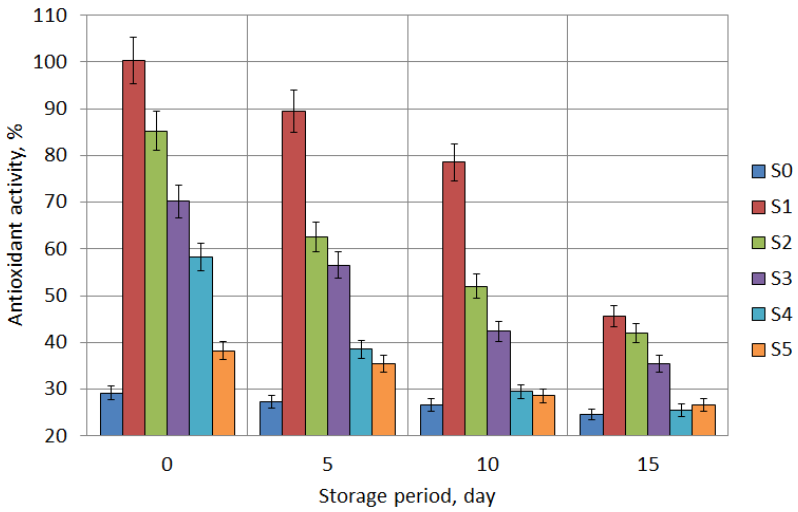


FIGURE 2. Antioxidant activity (AOA) of the beverage during storage

The values have statistically significant difference at p<0.05. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined.

The highest antioxidant activity was observed in sample S1 – 100.36%. and the lowest in the control S0. The high level of antioxidant activity in the samples is probably due to the content of ascorbic acid (vitamin C) in the beverage and the organic acids. flavonoids. pectins. tannins. etc. extracted during the extraction.

During storage. antioxidant activity decreases. and on the 15th day of storage it has the lowest values. According to Brykalov et al. [24]. the decrease in antioxidant activity in beverages is due to the high reactivity of polyphenolic compounds with amino acids. The pasteurization temperature of the beverages is also the reason for the overall decrease in antioxidant activity.

The active acidity of the obtained beverages was also monitored in the various hydromodules. both in the period of preparation and in its storage at 5. 10 and 15 days (Fig.3). The values of the active acidity of the beverages were compared with the values of the active acidity for pure whey S0.

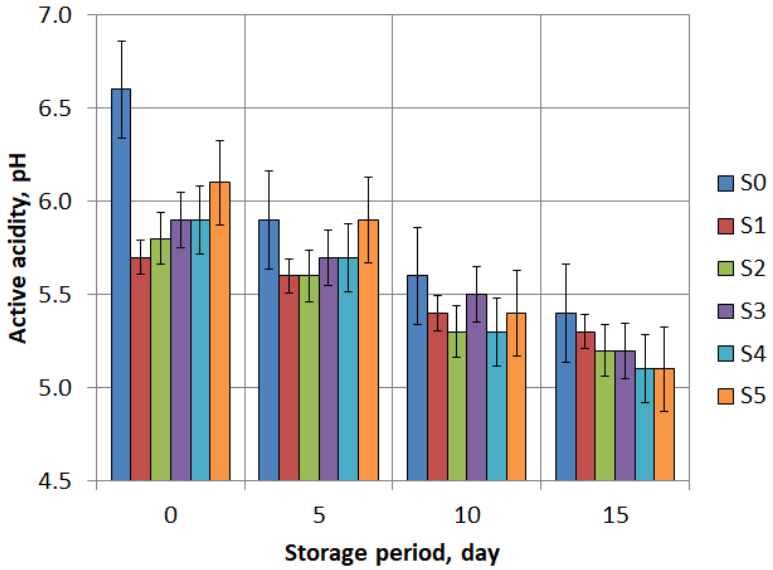


FIGURE 3. Active acidity of the beverage during storage

The values have statistically significant difference at p<0.05. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined.

In sample S1 on the first day of preparation of the beverage. the active acidity is 5.7 and decreases to 5.3 and on the 15th day of storage. The low values of the active acidity of the whey beverage in sample S1 are due to the biologically active substances and organic acids extracted from the rose hips. Similar results were observed in samples S2 and S3. with a decrease in the values of active acidity of about 10%. Similar results of lowering the active acidity have been obtained by other authors [25] in the preparation of a whey-based herbal drink.

The chemical composition of the obtained beverage during the storage period was analyzed.

The data in Table 7 show that in sample S1. the protein content was as in control S0. There is also a slight difference in protein in the other samples. which is probably due to the larger hydromodule at which the extraction process was carried out. Whey has been shown to contain the amino acids cysteine and methionine. which improve immune function [26]. The results obtained. as well as those reported in Rupnar et al. [8] proved that whey proteins are globular proteins that are soluble in a wide range of pH. therefore they are not affected by the extracted organic acids during extraction.

Table 7. Protein content of rosehip-whey drink during storage

Day Sample	0	5	10	15
S0	0.82±0.03	0.81±0.02	0.81±0.02	0.81±0.02
S1	0.82±0.03	0.82±0.03	0.82±0.04	0.82±0.04
S2	0.81±0.06	0.81±0.1	0.81±0.04	0.81±0.03
S3	0.8±0.1	0.8±0.03	0.8±0.01	0.8±0.02
S4	0.8±0.02	0.8±0.07	0.8±0.03	0.8±0.05
S5	0.8±0.06	0.8±0.02	0.8±0.04	0.8±0.04

The values have statistically significant difference at p<0.05. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

The lactose and fat content of all samples remained almost unchanged during the storage period. with a reduction of about 2 % (Tables 8 and 9).

Table 8. Fat content of rosehip-whey drink during storage

Day Sample	0	5	10	15
S0	0.2±0.01	0.2±0.02	0.2±0.1	0.2±0.04
S1	0.2±0.002	0.2±0.1	0.2±0.02	0.2±0.01
S2	0.1±0.1	0.1±0.02	0.1±0.03	0.1±0.01
S3	0.1±0.1	0.1±0.02	0.1±0.02	0.1±0.03
S4	0.1±0.03	0.1±0.03	0.1±0.02	0.1±0.02
S5	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.03

The values have statistically significant difference at p<0.05. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

Table 9. Lactose content of rosehip-whey drink during storage

Day Sample	0	5	10	15
S0	4.6±0.2	4.5±0.1	4.4±0.2	4.4±0.2
S1	4.6±0.1	4.5±0.1	4.4±0.1	4.4±0.1
S2	4.4±0.3	4.4±0.1	4.3±0.2	4.3±0.2
S3	4.3±0.1	4.2±0.1	4.2±0.1	4.2±0.1
S4	4.3±0.1	4.1±0.1	4.1±0.1	4±0.1
S5	4.2±0.2	4.1±0.1	4±0.1	4±0.1

The values have statistically significant difference at $p<0.05$. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

The total amount of minerals present in a normal dairy product sample is maintained. As can be seen from Table 10. the ash content of all samples remained almost unchanged during the storage period.

Table 10. Ash content of rosehip-whey drink during storage

Day Sample	0	5	10	15
S0	0.6±0.03	0.6±0.04	0.6±0.04	0.6±0.03
S1	0.8±0.02	0.8±0.03	0.8±0.03	0.8±0.03
S2	0.7±0.02	0.7±0.03	0.7±0.02	0.7±0.02
S3	0.7±0.03	0.7±0.03	0.7±0.03	0.7±0.03
S4	0.7±0.03	0.7±0.03	0.7±0.03	0.7±0.03
S5	0.6±0.02	0.6±0.03	0.6±0.02	0.6±0.02

The values have statistically significant difference at $p<0.05$. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

The dry matter content of the fortified whey was also determined. Table 11 shows that in sample S1 the dry matter content is the highest (6.42%). both on the first day of receipt and until the end of the storage period. The higher values are probably due to the extracted pectin and tannins during the extraction. For the other samples. the dry matter values range from 6.22 for sample S0 to 5.70 for sample S5. As the hydromodule increases. the dry matter values in all samples decrease slightly up to 15 days of storage.

Table 11. Dry matter of rosehip-whey drink during storage

Day / Sample	0	5	10	15
S0	6.22±0.04	6.1±0.1	6.1±0.1	6.1±0.1
S1	6.42±0.1	6.3±0.2	6.04±0.2	6.04±0.2
S2	6.01±0.2	6±0.1	5.73±0.1	5.73±0.2
S3	5.9±0.1	5.85±0.1	5.75±0.1	5.75±0.1
S4	5.9±0.04	5.9±0.03	5.8±0.3	5.7±0.3
S5	5.7±0.1	5.7±0.1	5.7±0.1	5.65±0.1

The values have statistically significant difference at $p<0.05$. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

Analyzes were made for the change of color and color indices of whey drink during storage and.

Figure 4 shows the color change of the samples for the entire storage period. The data show that the color of the product is preserved throughout the storage period. Additional analyzes are needed to determine the actual color change through color indices.

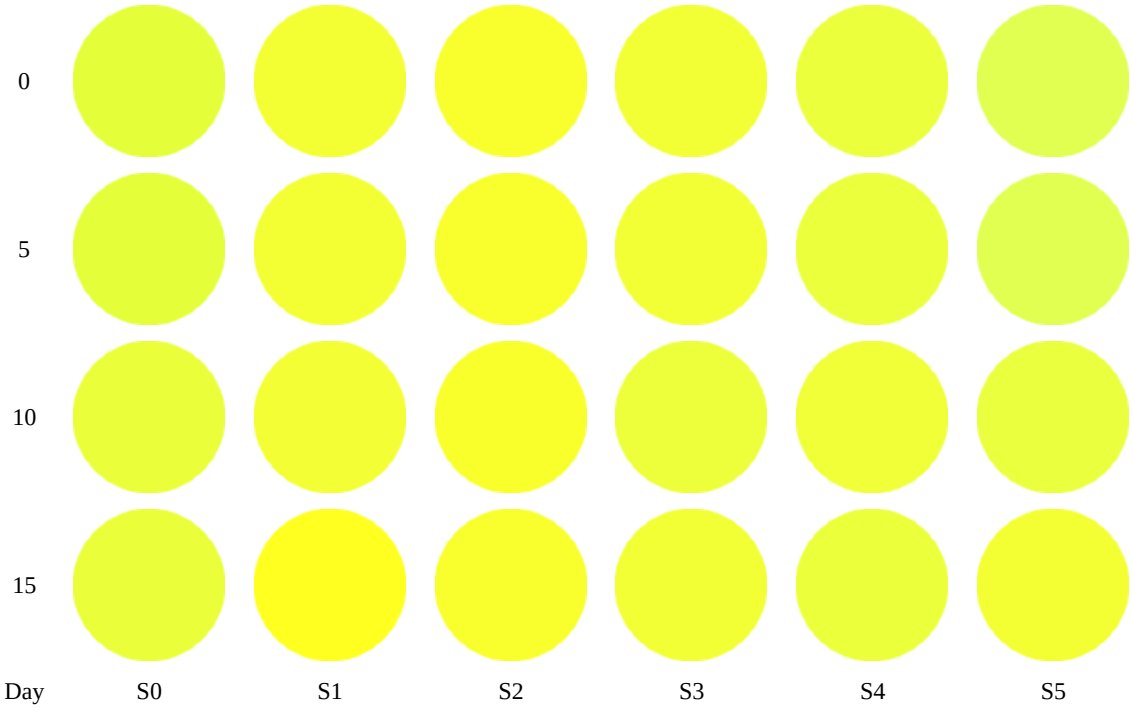
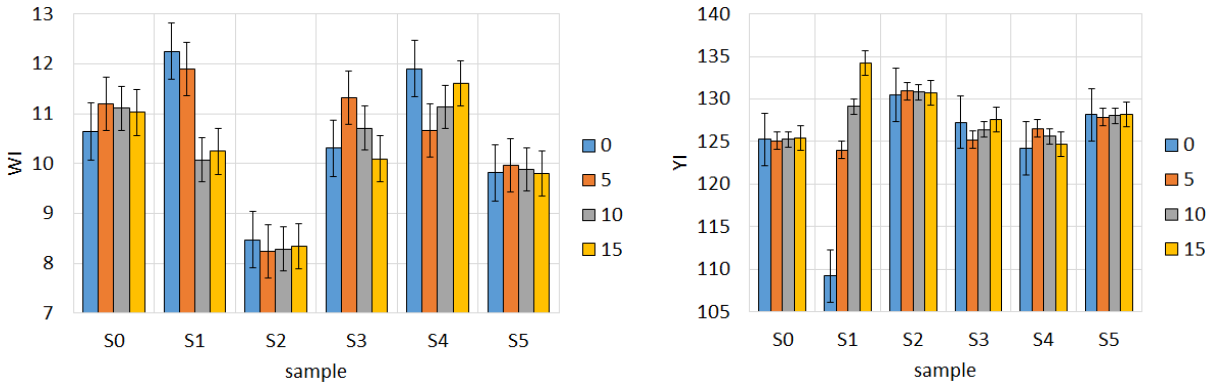


FIGURE 4. Color change of rosehip-whey drink during storage

When determining the organoleptic evaluation. the color of the beverage was measured spectrophotometrically. From the color obtained in the Lab color model. the yellow index (YI) and the white index (WI) are determined.

The yellow index remains constant in the various samples. The white index changes. similar to vitamin C and antioxidant activity. during storage. as shown in Figure 5. The results show that the drink retains its yellow-brown color throughout the storage period.



(a) Yellowness index (YI) (b) Whiteness index (WI)

FIGURE 5. Color indices of whey drink during storage
The values have statistically significant difference at p<0.05. All data were obtained in triplicate measurements. Their mean value and standard deviation were determined

Figure 6 shows the sensory profiles of the beverage during its storage period. The panel of tasters unanimously determined the beverage from sample S1 with the best indicators of color. smell. taste and appearance.

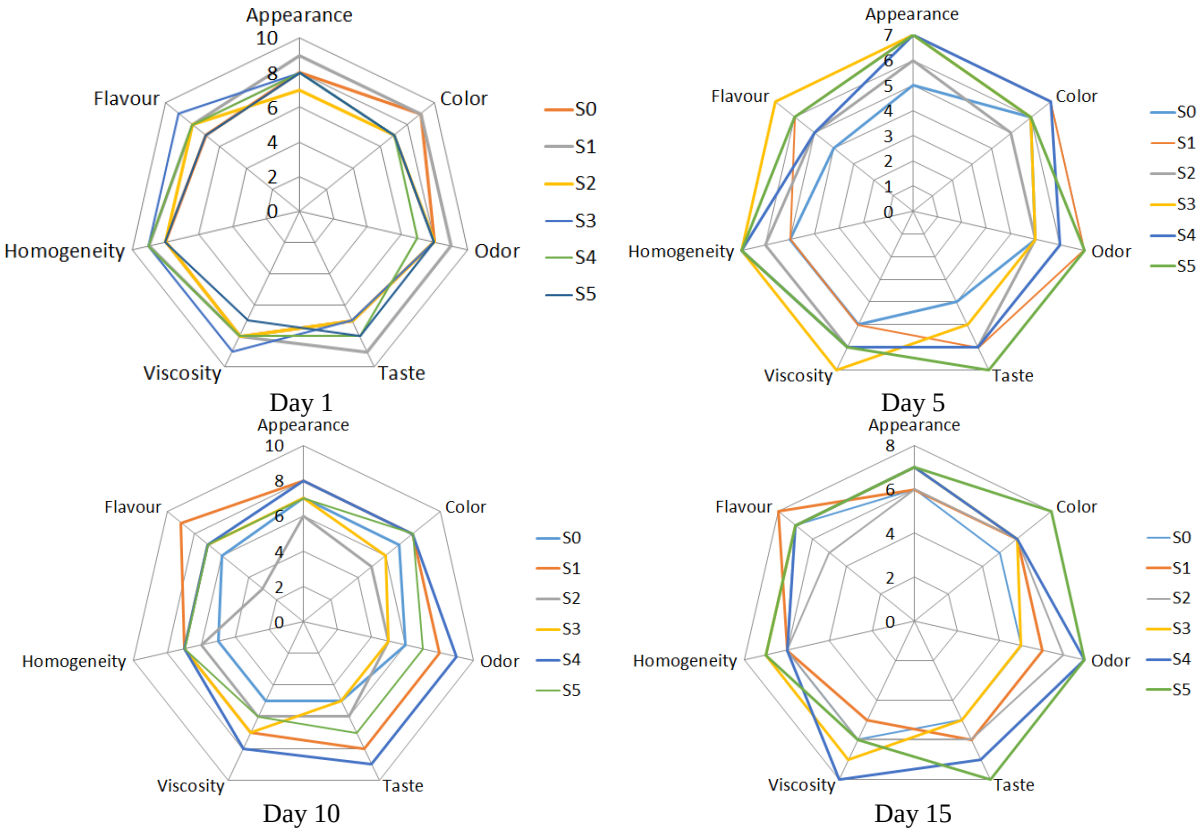


FIGURE 6. Sensory profile of whey beverage during storage

In order for a drink to be accepted by modern consumers. it must satisfy at least some of the basic consumer qualities – taste. smell. aroma. the effectiveness of quenching thirst. the favorable price and a positive effect on health.

In sample S0 during the storage period. the color indicator is not accepted by the tasters. A similar trend is observed in samples S1 and S2. The highest color ratings from the tasters were given to samples S4 and S5. compared to the others. In terms of appearance. aroma and taste throughout the storage period. the highest scores are given to samples S1 and S2. and in terms of homogeneity and viscosity. higher scores are given to samples S3. S4 and S5.

From the organoleptic analysis made. the commission of tasters unanimously determined the drink from sample S1 with the best organoleptic indicators.

A suitable hydromodule for the preparation of a whey drink with rosehip extract has been determined.

Those features that have weight coefficients above 0.9 were selected (Figure 7). This means that they depend to the greatest extent on the change of the hydraulic module. ORP was selected from the physico-chemical characteristics. and from the chemical ones – fats. ascorbic acid and antioxidant activity (AOA).

The optical and color characteristics A520 and A620. b and C also depend on the change of the hydraulic module. The color has the highest value of the weighting factor on the organoleptic characteristics.

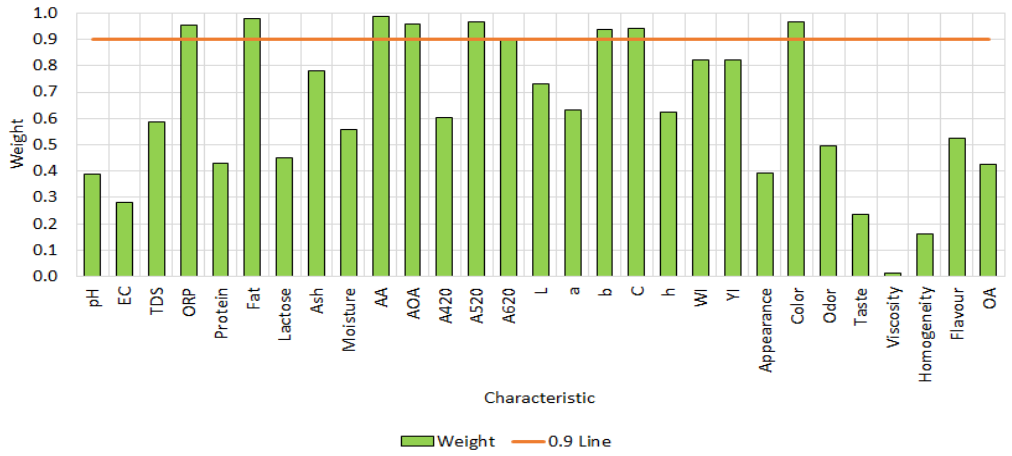


FIGURE 7. Results of Correspondence Analysis

Absorption at 520 nm (A_{520}) and fat were selected from all of the features. The predictive models of these two characteristics can be described with sufficient accuracy the connection with the hydromodule in which the drink was obtained. The other selected characteristics do not have this property. Of the selected features. the absorbance at 520 nm (A_{520}) is more important. It is in the visible region of the spectrum and refers to the yellow-green color of the drink.

A model was determined between the absorbance at 520 nm (A_{520}) and the hydromodule (H). it was used in compiling the simplex diagram. After removing the insignificant coefficients with $p>\alpha$. the model $A_{520}=f(H)$. has the form:

$$A_{520} = -4.89.10^5.H^3 + 0.0046.H^2 - 0.092.H + 2.43 \quad (3)$$

At F (2.15)=3.43<Fcr (2.15)=3.68 and R^2=0.67. Standard error SE=0.11. The model can be considered to describe the relationship between the dependent and independent variables with sufficient accuracy. Analysis of the residues of the obtained model shows that it is adequate. When analyzing the residues. it is established that there are no systematic deviations of the actual data from the theoretical ones. which is a sign of their normal distribution.

Figure 8 shows the determined suitable hydromodule. by means of a Simplex diagram. Along the horizontal axis. the hydromodule is plotted. The percentage of fat is plotted on the left vertical axis. On the right vertical axis is the absorption at 520 nm.

The composition-characteristic diagram reflects the influence of the hydromodule on the selected characteristics of the milk drink. The simplex isolines of the diagram reflect all the coordinates of the experimental-statistical lattice. for which the considered characteristic of the drink has the same values. Each point of the simplex is completely defined. which means that for each point of the diagram a hydromodule can be determined. at which the predicted value for a specific qualitative characteristic of the beverage is obtained.

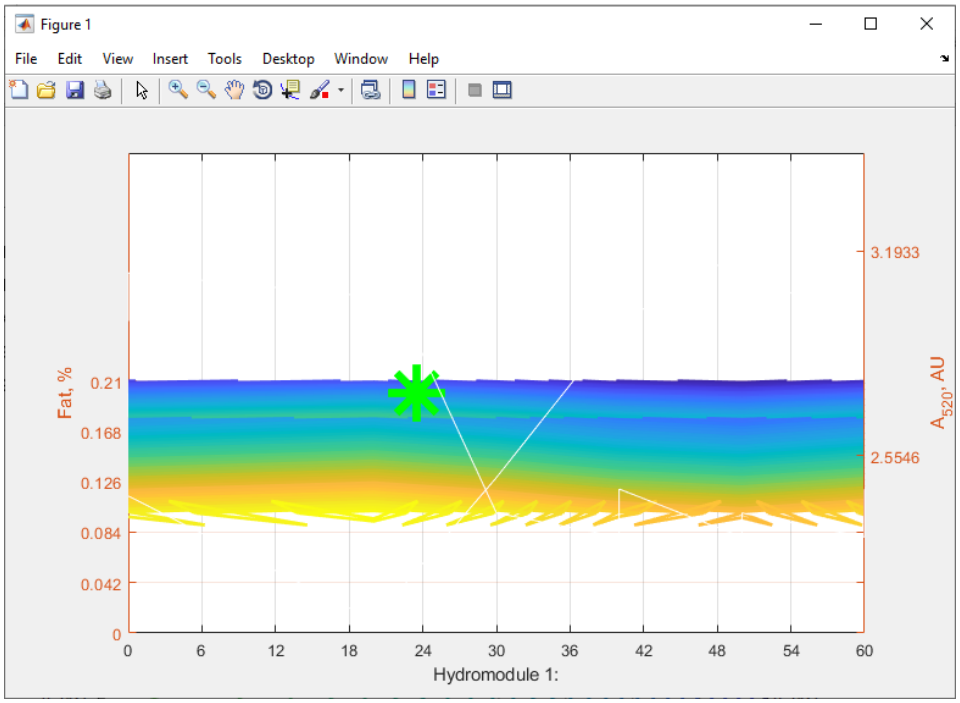


FIGURE 8. Simplex diagram of Hydromodule-Fat-Absorption

From the calculations made and the simplex diagram. a suitable hydromodule was determined. which is 1:23.47. The results obtained are confirmed both by the color feature analysis described above and by the organoleptic analysis. in which high values of the total organoleptic evaluation are obtained at hydromodule 1:20.

Hydromodule 1:24 was selected as suitable for producing a whey beverage that has acceptable physicochemical. organoleptic and optical properties.

CONCLUSION

From the results of this study it can be concluded that the whey enriched with biologically active substances. through the extraction process can be prepared and stored without loss of quality at low temperatures 0-4 °C.

The best indicators for enriched whey (high AOA and vitamin C content) are observed in sample S1. ie. sample obtained at hydromodule 1:20. temperature 0-4 °C; extraction duration 3 h. With this hydromodule. the main indicators of whey. such as protein. lactose. fat content and dry matter. are most fully preserved.

Computer-based method and tools for automated determination of a suitable hydromodule for preparation of whey drink and rosehip extract have been adapted and studied. After refining the data obtained. it was found that a suitable hydromodule is 1:24. When using this hydromodule. the physico-chemical and organoleptic properties of the beverage are not disturbed.

It was found that the spectral absorption characteristics obtained in the range of 520 nm and the amount of fat are a suitable tool for analysis of the proposed beverage.

In obtaining the drinks. waste raw material from cheese-making was used. which would reduce costs in this industry. In addition. the consumption of the resulting drinks. which can be considered as functional drinks. have a positive impact on the health of consumers. as well as to solve health problems in everyday active life.

The extraction method can be used in the development of new functional whey-based beverages with other fruits. in order to increase its functional and nutritional qualities.

ACKNOWLEDGEMENTS

The work in this paper is partially supported by project № 3.FTT/2021 with topic "Ultrasonic extraction of dill (*Anethum graveolens*) and white oregano (*Origanum vulgare* L.)"

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