The development of a functional adaptogenic beverage, using plant extracts of Centella asiatica and Hoodia gordonii

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Abstract. A functional beverage of adaptogenic action based on plant raw materials was developed using dry plant extracts of Centella asiatica and Hoodia gordonii to impart functional and biologically active properties. An organoleptic analysis, namely, the free profiling method, was used to compose the beverage recipe. Based on the constructed profilograms, the optimal ratio of the blend of juices and teas, which is 1: 0.65, was chosen. In beverage, the basic physicochemical parameters of the finished functional drink are determined, such as: solids content, total (titratable) acidity, hydrogen index (pH) of coloring substances (anthocyanins), macronutrients (Na, K, Mg, Ca) and trace elements (Fe, Cu, Zn, Mn). In accordance with the requirements of the technical regulation of Customs Union 021 "On Food Safety", the adaptogenic beverage was tested for the content of toxic elements: arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg). As a result, the content of toxic elements doesn’t exceed the maximum permissible concentration. In the scientific work, we determined the shelf life of a functional beverage of adaptogenic and anti-alcohol action. Using the accelerated shelf life testing method (ASLT), which is based on the Arrhenius model, we determined the shelf life of the beverage = 3.7 months.

1. Introduction

Over the past decade, interest in a healthy lifestyle has significantly increased, especially among populations leading socially active lifestyles. New approaches to choosing a diet are being formed, aimed at strengthening the body, reducing the risks of various diseases, and increasing overall tone. In this regard, food manufacturers set themselves the task of creating new functional products enriched with natural substances, micro-, macroelements and vitamins, biologically active ingredients, which are designed for target consumer groups and for certain body systems [1, 2].

A functional product is a product with beneficial nutritional characteristics that meet the requirements of specific populations. The functional product is intended for systematic use in food rations. It has scientifically based and confirmed properties that reduce the risk of developing diseases, regulate certain processes in the body due to the presence of functional food ingredients in their composition. The main components of functional products are nutritional value, pleasant taste, positive physiological effect.

1.1. The market of functional beverages

The liquid form of transport of nutrients and biologically active substances into the body is the most practical and is quickly absorbed in the gastrointestinal tract (within 15-20 minutes), which gives a clear advantage to a functional beverages among other products. The market of functional beverages is
one of the fastest growing. Mordor Intelligence has predicted that by 2024 the functional beverage market will reach 208.13 billion US dollars. In the forecast period (2019-2024), the CAGR (Compound Annual Growth Rate) is expected to be 8.66% [3]. Such demand is also caused by a change in consumer preferences. They began to prefer functional beverages, rather than fruit juices and carbonated beverages. Also, increasing awareness of the proper nutrition and composition of food products contributes to an increase in the consumption of functional foods.

Currently available functional beverages for special purposes contribute to solving a number of problems related to the prevention and reduction of the risk of diseases of the cardiovascular, immune, nutritional nature, etc. However, there are no functional beverages on the market aimed at reducing the risks of brain diseases and having adaptogenic effects, as well as reducing the effects of alcohol on the brain, having nootropic and anxiolytic effects. According to the World Health Organization (WHO), 2.5 million people with alcohol dependence are registered in Russia, and this indicator is growing steadily. Excessive alcohol consumption often leads to chronic alcohol intoxication (alcoholism) and is accompanied by functional changes in the brain, provoking a further increase in craving for alcohol.

In addition, WHO predicted that in 2020, brain diseases and mental disorders will be among the top five diseases leading to disability. In Russia, more than 20% of people suffer from neurotic disorders. 75-89% of all neurological diseases are disorders of memory, personality, depression, anxiety, psychosis, and depression.

In this regard, it seems promising to develop a functional beverage with adaptogenic and anti-alcoholic effects.

Adaptogenic action is an increase in the general resistance of the body to the action of extreme factors. Compounds with a pronounced adaptogenic effect also have a mnemotropic effect (stimulation of learning and memory), anti-asthenic action (a decrease in the severity of weakness and lethargy), psychostimulating (inhibition of mental inactivity and apathy) and anxiolytic effect (anti-anxiety, sedative). The relevance of such beverages is due to the fact that the level of diseases associated with disorders of the nervous system and brain activity is increasing, the number of people suffering from alcohol dependence is increasing. Negative environmental factors affecting the human body, as well as a large amount of information processed by the brain, cause increased fatigue, decreased concentration, nervous disorders, stress, lethargy, fatigue and much more.

To give the products functional properties, manufacturers are increasingly resorting to the use of plant extracts of fruits, berries, herbs. Among a large number, we selected two plant extracts: Centella asiatica and Hoodia gordonii, which have the properties we need.

1.2. Hoodia gordonii extract

A number of studies show the effectiveness of the plant extract of Hoodia gordonii (H. gordonii), containing the substance P57AS3 (P57), to suppress appetite [4, 5, 7]. The mechanism of pathological attraction to food (obesity) and alcohol (alcoholism) is realized with the participation of the hormone gastrointestinal tract ghrelin, which directly affects the center of hunger and saturation of the hypothalamus - an element of the limbic system of the brain. The concentration of the hormone ghrelin increases both during hunger and during the period of alcohol withdrawal (withdrawal period). Therefore, it can be assumed that H. gordonii extract is able to indirectly reduce the level of pathological craving for alcohol. The mechanism of its action remains to be studied in depth, since the available information is insufficient.

The relationship between ghrelin and the development of alcohol dependence was previously established by Elizabeth Yerlag of the University of Gothenburg. Researchers have been able to prevent the development of alcoholism in mice by blocking the signal for “hunger hormone” [4].

It has been proven that the greatest amount of ghrelin is released during the period of giving up alcohol, which is why people who are in a state of a hangover begin to drink alcohol again. Studies at the Scripps Institute have proven that ghrelin stimulates the activity of neurons responsible for the development of alcohol dependence.
The effect of H. gordonii was studied in vivo in rats and birds and is confirmed by our rodent studies at the Institute of Experimental Medicine [4, 7, 8] (results not yet published).

1.3. Centella asiatica Extract
Centella asiatica extract stimulates the release of acetylcholine, which regulates the signal transmission speed in synaptic nodes, has a sedative effect, improves brain cognitive functions due to saponins (complex nitrogen-free organic compounds, glycosidic nature). Biologically active substances of Centella are asiatic and madecassonic acids. A 500 mg dose of Centella Asiatic has been studied twice daily to reduce anxiety in humans by binding to cholecystokinin receptors [9].

One of the best-known benefits of Centella Asiatica is its ability to improve cognitive function and strengthen memory.

A study published in the Journal of Alzheimer’s Disease showed that Centella Asiatica Extract can improve memory at the molecular level, increasing the activity of pathways associated with the formation of long-term memory. In a 2002 study in animals, where male Wistar rats weighing 200-250 g were used to study the effects on learning and memory [10]. They received an aqueous solution of Centella Asiatica extract and underwent a series of tests aimed at evaluating cognitively stimulating functions. It has been found that the extract improves learning and memory ability by increasing the level of antioxidants in the blood [10, 13].

In a 2010 study of the anxiolytic property of Centella Asiatica extract using a Hamilton scale to evaluate depression, 33 people participated. For two months, they received 500 milligrams of Centella Asiatica extract twice a day. As a result, a decrease was found not only in the level of anxiety, but also in the level of stress and depression [11, 12].

Other studies published in the journal Journal of Ethnopharmacology have come to similar conclusions. They demonstrated that elderly subjects who received Centella Asiatica extract experienced an improvement in mood and memory [12-14].

Thus, the functional properties of the extracts used are proven and studied.

2. Materials and methods

2.1. Materials
To prepare the beverage, we used plant raw materials:
- direct-pressed juices from the fruits of red grapes (Red Globe), apples (Red Delicious, Granny Smith), blueberries (Ordinary), carrots (Queen of the Autumn), kiwi (Abbot);
- two kinds of tea: black Ceylon fermented, green Chinese unfermented;
- dry plant extracts were used as functional ingredients: Centella asiatica, Hoodia gordonii (H. gordonii).

All ingredients in the recipe are selected taking into account the chemical composition, namely the presence of a large number of flavonoids (and other compounds of a glycosidic nature, coloring matter of eotanins and anthocyaons), saponins (asiatic acid, madecassonic acid, asiaticoside and madecassoside), which have powerful nootropic effect (stimulation of intellectual activity and neurometabolism) [15]. In addition, saponins of Centella asiatica extract in combination with glycosides, such as P57AS3 extract of H. gordonii, provide a complex synergistic effect on the brain and limbic system.

2.2. Methods

2.2.1. Organoleptic methods. When developing a functional beverage, organoleptic and instrumental methods of analysis were used.
To develop the recipe for the beverage, we used the method of testing organoleptic properties - free profiling. One of the important advantages of this method is that during the study it is easy to include new properties that appear during the experiment in the compiled profile. Based on the profilograms, the ratio of the components of the beverage was determined. The assessment was made according to 6 indicators: sweet taste; sour taste; aroma; color; aftertaste; bitterness. A 3-point scale was used (1 - bad, 2 - good, 3 - excellent) [16, 17].

2.2.2. Physiochemical properties of a functional beverage:
- The determination of dry substances - the method is based on the phenomenon of total internal reflection of light from a radiation source at the interface between two media. A PTR 46 refractometer (Index Instruments Ltd., United Kingdom) was used;
- The determination of total acidity by the titrimetric method - the method is based on the ability of acids to neutralize with alkali. An 848 Titrino plus titrator (Metrohm, Switzerland) was used;
- The determination of pH - active acidity by the potentiometric method - the method is based on measuring the electromotive force of a galvanic circuit. An 848 Titrino plus titrator (Metrohm, Switzerland) was used;
- The determination of the content of coloring substances (flavonoids: anthocyanins) by the photoelectrocolorimetric method. The principle of operation is based on the determination of optical density at a wavelength of 530 nm using a cuvette with a wall width of 1 mm on a KFK-3-01 instrument (ZOMZ, Russia).

\[ C = K \cdot D, \]

where \( C \) is the concentration of coloring substances, mg / dm\(^3\);
\( D \) is the optical density;
\( K \) is the conversion factor for malvidin, 1056.7.

2.2.3. Determination of the content of toxic and mineral elements with preliminary sample preparation
- The sample preparation for determination of heavy metals was carried out by the method of wet mineralization on a thermal installation EHD 36 LABTECH at a temperature of 150–180ºC (Italy).
- The measurement of arsenic (As) was carried out on a Shimadzu AA-6300 atomic absorption spectrophotometer with the type of atomization — hydride generation (Japan).
- Cadmium (Cd) and lead (Pb) were analyzed on a Shimadzu AA-6300 atomic absorption spectrophotometer with electrothermal atomization (Japan).
- Mercury (Hg) was measured on a Julia 5-K instrument using the cold method (Russia).
- The mineral composition of the functional beverage was carried out by spectral analysis on a Shimadzu AA-6300 spectrophotometer with a type of flame atomization (Japan). The trace elements — Fe; Cu; Zn; Mn and macronutrients - Na; K; Mg; Ca.

2.2.4. Determination of the shelf life of a functional beverage using the method of accelerated shelf life testing. We used the Accelerated Shelf-Life Testing (ASLT) method based on the Arrhenius model and reflecting the dependence of temperature on the rate of a chemical reaction (rate of deterioration of product quality).

\[ K = K_0 \cdot \exp \left( \frac{-E_a}{RT} \right) \]

where \( K_0 \) is a constant;
\( E_a \) - activation energy;
\( R \) is the gas constant;
\( T \) is the absolute temperature.
We proceeded from the assertion that an increase in the standard temperature (20 °C) for every 10 °C increases the speed of the chemical reaction, and therefore, worsens the quality of the product 2 times.

The process was controlled by changing the content of anthocyanins at a temperature of 40 and 50°C.

The end of the test time was considered the period when the functional beverage became unsuitable for a controlled parameter (change in the content of one of the functional components, in this situation: anthocyanins). The test was carried out in a heat chamber [17, 18].

3. Results and Discussion

The development of a functional beverage by the organoleptic method. The method of free profiling was used to select the optimal ratio of the blend of juice directly pressed and tea infusions, presented in table 1.

Table 1. The composition of functional beverage

| The name of ingredient | The amount in the finished beverage, gram |
|------------------------|------------------------------------------|
| Red grape juice        | 18.0                                     |
| Carrot juice           | 12.6                                     |
| Kiwi juice             | 12.6                                     |
| Blueberry juice        | 9.0                                      |
| Apple juice            | 9.0                                      |
| Black tea              | 30.0                                     |
| Green tea              | 8.4                                      |
| Centella Asiatica dry extract | 0.25                      |
| Hoodia Gordonii dry extract | 0.06                                    |

We took various ratios of blends of juices of direct extraction and tea infusions (Figures 1, 2, 3). The assessment was made according to 6 indicators: sweet taste; sour taste; aroma; color; aftertaste; bitterness. We used a 3-point scale (1 - bad, 2 - good, 3 - excellent).

Then organoleptic tastings were carried out and tasting sheets were compiled, on the basis of which the optimal profile was selected, which corresponded to the best organoleptic characteristics of a functional beverage [16].

The profile is shown in Figure 2. The optimal ratio of the blend of direct-squeezed juice and tea infusions is 1: 0.65.

Figure 1. Blend Ratio 1: 1
Figures 1 and 3 show that the beverage is not balanced and has a bitterness and an unpleasant aftertaste. In addition, in Figure 3, a 1:0.5 ratio demonstrates a weak balance of sweet and sour taste.

Figure 2 shows that all quality indicators of the finished beverage in harmony, which gives the right to use it as a reference sample.

The resulting functional beverage has a balanced sweet and sour taste, a pleasant, characteristic aroma used by raw materials, with light fruity unobtrusive notes, as well as a pleasant astringency of green tea. The purple-red color of the beverage is due to the content of anthocyanins in the raw material. The beverage has harmonious organoleptic properties.

*Extracts.*

Hoodia Gordonii and Centella Asiatica plant extracts were added to the formulation to ensure functional properties.

The functional blend of dry plant extracts was based on daily and recommended consumption doses to achieve the desired effect. Thus, Centella Asiatica extract was added in an amount of 25% (250 milligrams) of a daily dose of 1000 milligrams [9]. Hoodia Gordonii extract was added in an amount of 15% (60 milligrams) of a daily dose of 400 milligrams [20, 21].

Next, the physicochemical properties of a functional beverage of adaptogenic action were determined. The results are presented in table 2.

Next, the content of toxic elements was determined. The finished beverage was tested for the content of the safety indicator - toxic elements according to the technical regulation of the customs union 021/2011 "On food safety". The results are presented in table 3.
Table 2. Physiochemical properties of a functional beverage

| Indicator                          | Indicator value |
|-----------------------------------|-----------------|
| PH value                          | 3.8             |
| Titratable acidity, degrees      | 5.7             |
| Solids content, %                 | 7.7             |
| The content of anthocyanins mg / l| 573.4           |

Table 3. The content of toxic elements in a functional beverage.

| The element     | Result                  | The normalized content |
|-----------------|-------------------------|------------------------|
| Arsenic (As)    | less than 0.05 mg / l   | no more than 0.1 mg / l|
| Cadmium (Cd)    | less than 0.02 mg / l   | no more than 0.03 mg / l|
| Lead (Pb)       | less than 0.2 mg / l    | no more than 0.3 mg / l|
| Mercury (Hg)    | less than 0.01 mg / l   | no more than 0.05 mg / l|

The content of toxic elements meets the requirements of technical regulations and does not exceed the maximum permissible concentration (MPC).

The mineral composition of a functional beverage was determined too. The mineral composition has a complex synergistic effect on the brain system in combination with plant extracts Centella asiatica, Hoodia gordonii and flavonoids [22, 23]. The results are presented in table 4.

Table 4. The content of macronutrients and trace elements in a functional beverage.

| The element | Concentration in the sample, mg / dm³ | Daily norms of mineral elements, mg |
|-------------|---------------------------------------|-----------------------------------|
| Na          | 28.19 ± 0.2                           | 550                               |
| K           | 1207.00 ± 0.35                         | 2000                              |
| Mg          | 47.35 ± 0.5                            | 350                               |
| Ca          | 555.97 ± 0.25                          | 1000                              |
| Fe          | 0.56 ± 0.1                             | 10                                |
| Cu          | 0.42 ± 0.15                            | 1.0–1.5                           |
| Zn          | 0.60 ± 0.2                             | 10                                |
| Mn          | 2.51 ± 0.4                             | 2–5                               |

The shelf life of the developed functional beverage was determined by the accelerated testing method at temperatures of 40 and 50 °C. The result was determined as the arithmetic mean of two parallel measurements [24].

The control sample was in a refrigerator at a temperature of 20 °C. The dependences of the concentrations of anthocyanins as an indicator of functionality on the exposure time at temperatures of 40 and 50°C are presented in Figures 4 and 5.
Figure 4. The dependence of the content of anthocyanins on the exposure time at 40 degrees Celsius

Figure 5. The dependence of the content of anthocyanins on the exposure time at 50 degrees Celsius

The experiment was stopped when the degradation of anthocyanins reached 35%. Thus, at 40 degrees Celsius and at 50 degrees Celsius, the experiment was completed on days 27 and 14, respectively [18].

At a temperature of 40 degrees Celsius: \(27 \times 2 \times 2/30 = 3.60\) months.
At a temperature of 50 degrees Celsius \(14 \times 2 \times 2 \times 2/30 = 3.73\) months.

The shelf life of the resulting functional beverage was \(~ 3.7\) months.

4. Conclusion

The formulation of a prophylactic functional beverage based on plant raw materials and plant extracts of H. Gordonii and Centella Asiatica has been developed, which have an adaptogenic effect and reduce craving for alcohol.

Using the free profiling method, the optimal ratio of the components of this beverage was selected. It is 1: 0.65.

Physicochemical parameters were measured, such as: solids content, total acidity, pH active pH, coloring matter: anthocyanins, as an indicator of functionality.

The mineral composition of the functional beverage was determined, namely, the content of macroelements (Na, K, Ca, Mg) and microelements (Fe, Cu, Zn, Mn), which provide a complex synergistic effect on the brain in combination with plant extracts of H. Gordonii and Centella Asiatica.
It has been established that the developed beverage is safe by one of the main safety indicators, namely, by the content of toxic elements (As, Cd, Pb, Hg). As a result, the data obtained don’t exceed the maximum permissible concentrations.

The shelf life of a functional beverage was determined by the ASLT - accelerated test method at temperatures of 40 and 50 degrees Celsius. The shelf life of a functional adaptogenic beverage is ~ 3.7 months.

The developed functional beverage can be recommended for people who are subject to excessive stress and anxiety. The beverage is intended for people suffering from alcohol abuse. Also, the beverage is recommended for people who have a strong information load and are engaged in active intellectual activities that require concentration, attention and a good memory.

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