Whey beverage with collagen hydrolysate from salmon skin and feijoa

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Abstract. The article focuses on the development of a functional beverage produced from cheese whey and collagen hydrolysate being wastes of dairy and fishing industries. Fish collagen hydrolysate was hydrothermally extracted from salmon skin. Further, the sample was cooled, frozen and vacuum freeze dried. Collagen content in the obtained hydrolysate was equal to 37.5%. The hydrolysate was added to the whey beverage in the dose of 0.25%, 0.5%, 0.75% and 1%. In order to reduce the smell and taste of fish, 0.5% of feijoa puree was added to the beverage. In the beverages active acidity, titratable acidity, antioxidant activity and sensory parameters were measured. It is shown that the most rational dosage of collagen hydrolysate in beverages was 0.5%. The beverage had a clean, whey taste with a pleasant hint of feijoa, without any foreign flavours or odours. Consistency of the whey beverage was even and liquid, the colour is uniformly white, which is common for whey, with a hint of feijoa. The active and titratable acidity of the selected beverage were at 4.5 and 60 °T respectively. Total antioxidant activity of the beverage was 0.47 mg/g of dry substance

1. Introduction
Disposal of wastes generated by food-handling establishments is a huge problem for almost all regions of the Russian Federation. For this reason, the food industry is tasked with complex processing of wastes. Thus, for dairy industry, advanced processing of milk includes a rational usage of all its components, including whey used as a waste. The relevance of this national problem is reflected in the benchmarks of the Dairy Industry Development Program of the Russian Federation for 2013 – 2020 in the form of the "Use of milk whey for food purposes" index. It is well-known that milk whey is the best source of high quality protein. Milk whey proteins (lactalbumin, lactoglobulin and immunoglobulin) have the highest fission rate among whole proteins. Amino acid profile of whey proteins is the one closest to human muscular tissues and in the amount of essential amino acids and branched-chain amino acids (BCAA) - valine, leucine and isoleucine - they are superior to all other animal and plant proteins [1]. Besides, milk whey proteins significantly lower blood cholesterol level. Digestibility of the whey proteins is exceptionally high [2]. In addition, whey proteins provide an advantage against a wide variety of metabolic diseases, such as cardiovascular accidents, hypertension, obesity, diabetes and cancer [3, 4]. Studies show that whey protein is essential for faster trauma recovery, stimulation of the gastrointestinal tract and skin protection from harmful radiation. Whey protein is used to improve the nutritional value of enriched food, such as beverages
and soups and as a nutritional supplement for people, who need a higher daily protein consumption, for example sportsmen and senior people [5, 6].

For fish-processing industry, wastes containing collagen (skin, fins, bones) can serve as excellent, but underutilized protein source, they contain high quality proteins and lipids and also from 10 to 18 thousand chemical species, many of which are known to be pharmacologically active [7]. While the generation of wastes from hydrobionts processing is inevitable, fish-processing and biotechnological industries of the Russian Federation are tasked with deep processing of aquatic biological resources (Government Program of the Russian Federation “Development of Fisheries Industry”, 2014; "Complex Development Program for Biotechnologies in the Russian Federation for the period until 2020", 2012), it is related to the necessity of solving nature protection problems and obtaining value-added products.

Fish skin contains large amounts of collagen, which possesses a specific amino acid profile with high level of glycine, proline and hydroxyproline. Upon ingestion, collagen is not completely dissociated to the amino acids which, along with low molecular weight and particle size, allows it to be absorbed through the gut wall and distributed throughout the body via the bloodstream. Due to the fact that biochemical composition of marine collagen is highly similar to human collagen (up to 96%), cells may be stimulated to synthesise collagen in joint tissue, bones, derma and other body systems [8, 9].

Hydrolyzed proteins of marine collagen have the greatest value. During the decomposition process collagen is divided into separated peptides with low molecular weight and consequently, they enter the cell membranes faster, which allows to classify collagen supplements as polyfunctional components. During transition to gluten and gelatin, collagen can act as a dietary fiber, contributing to metabolic processes and having a positive impact on the condition and functioning of human gastrointestinal microbiota. Due to those characteristics, hydrolyzed proteins of fish collagen can be promising, if used in dairy products for bacterial population control, cartilaginous and connective tissue recovery and coincidently, for achieving the necessary product structure.

The aim of the researches was to develop a beverage, produced from some valuable wastes of dairy and fish-processing industries and to research its properties.

2. Materials and methods

2.1. Production of fish protein hydrolysate
The fish protein hydrolysates produced from Salmon (Salmo salar) skin prepared by hydrothermal method. This method included the rinsing of raw fish material and heat treatment (80°C) in a cheese whey medium at a ratio of 1: 3 for 60 minutes. This was followed by the separation of the liquid phase, with the solid fraction placed in a clean whey solution and re-treated. The liquid phase was cooled and degreased by centrifugation. The isolation was carried out three times. Then the liquid phases were mixed, cooled and vacuum freeze dried.

2.2. Determination of collagen content
Collagen content in the freeze-dried hydrolysates was determined by the method of Neuman and Logan and expressed as collagen content percent of dry substance. The method is based on hydroxyproline content of acid hydrolysis of the sample product, conduction of color reaction with its oxidative products and measurement of the occurring coloration intensity. For coloration measurement Specord M40 spectrophotometer was used. In order to determine the collagen content, the data received on hydroxyproline content had to be corrected according to the standard for collagen containing products (% of protein fractions total content), for which a special factor of 7.63 (for collagen) was used.
2.3. Beverage preparation
Freeze-dried feijoa puree was added in the beverage to reduce the distinctive smell of fish from collagen hydrolysate. Feijoa fruits were previously peeled and pureed, frozen and freeze dried. Dry cheese whey, restored to the dry solids concentration of 6.0%, was used as the basis for the beverage. Freeze-dried powder of feijoa (0.5%) was added to the restored cheese whey, that is equivalent to 5% of fresh feijoa puree. The content of freeze-dried feijoa powder was determined during the preliminary research stage [10], and was based on the results of sensory evaluation of whey beverage with feijoa. The collagen hydrolysate of 0.25%, 0.5%, 0.75%, and 1.0% was also added. The obtained samples of whey beverage were homogenized and pasteurized at 85-87 °C with constant stirring. Then the samples of the whey beverage were cooled to 4±2 °C and held at that temperature for 12 hours. The whey beverage, containing 0.5% of freeze-dried feijoa powder, without fish protein hydrolysate was used as the control sample.

2.4. Sensory evaluation
Sensory evaluation of the products was carried out for the following parameters: colour, taste, aroma, consistency and overall acceptance. The control was the traditional whey beverage prepared without fish hydrolysates. The panelists evaluated each attribute on a ten-point scale against hydrolysate-free whey beverage. Each panellist received individually about 15-20 ml of the control and researched samples.

2.5. Determination of active acidity
Active acidity was defined by a potentiometric method using Seven Go Duo pH Meter (Mettler Toledo).

2.6. Determination of titratable acidity
The titratable acidity (TA) of the whey beverages was measured using a digital burette Biohit Biotrate (Sartorius AG, Finland). The TA was expressed as percentage lactic acid.

2.7. Determination of Antioxidant Activity
Evaluation of the antioxidant activity was performed by coulometric titration with electrogenerated bromine using an EKSPERT 006 coulometric analyzer (EkoniksEkspert, Moscow) with glassy carbon electrodes [11]. The reference (anode of was 2.3 cm² in area) and the auxiliary (cathode) electrodes were glassy carbon rods of 3 mm in diameter; needlelike platinum electrodes were the indicators; 0.2 M potassium bromide in 0.1M sulphuric acid was the supporting electrolyte; the operating current was 5.27 mA, the auxiliary current was 0.79 mA; the level of measurement was 300 mV, the level of reduction was 500mV. The cathode and anode compartments were separated by a semi-permeable membrane. The supporting electrolyte (30 mL) was placed into a coulometric cell, then reference, auxiliary, and indicator electrodes were dipped into the electrolyte. The cell was placed on a magnetic stirrer to maintain constant stirring throughout the experiment. The coulometric analyzer was operated as per the manufacturer’s instructions. The antioxidant content of the samples was calculated according to Faraday’s law using the built-in software. Each sample was analyzed five times and the average value calculated.

2.8. Statistical Analysis
The results are presented as the values ± Standard Deviation (SD). Tukey’s test (p<0.05) (Bower, 2009) was used to detect significant differences between treatments. P-values below 0.05 were considered significant.
3. Results and discussion

3.1. Researches of hydrolysate properties

Hydrolysis is one of the ways to produce protein products from low-value raw materials, which allows obtaining preparations of isolated collagen proteins of high purity, as well as stimulation of the formation of the most important functional and technological properties in the context of the food industry branches, in particular, in the context of dairy products production. Using the hydrothermal method, a fine powder with a colour ranging from white to cream-white with a particle size of up to 500 μm and a slightly fish odour was obtained. Collagen content in the obtained hydrolysate was equal to 37.5%. The obtained hydrolysate was added to the beverage.

3.2. Sensory evaluation of the beverage

The results of sensory evaluation of researched beverages are provided on figure 1.

![Figure 1](image)

The maximum total number of points (29) was obtained by the whey beverage, containing 0.5% of freeze-dried collagen hydrolysate. The beverage had a clean, whey taste with a pleasant aroma of feijoa, without any foreign flavours or odours. The consistency of the whey beverage was even and liquid with moderate sediment from feijoa pulp, the colour was uniform, white, with a shade of characteristic feijoa colour.

3.3. Active and Titratable acidity

The results of the active acidity of the researched beverages are presented on figure 2 and titratable acidity is on figure 3.

![Figure 2](image)

Active acidity the beverage without the addition of collagen hydrolysate (control) was 4.43. Since the researched collagen hydrolysate had a neutral acidity and was added no more than 1%, the values of active acidity increased slightly to 4.52.
Figure 3 shows that an increasing of the content of hydrolysate decreases the titratable acidity value of the whey beverage from 64 °T to 58 °T.

Thus, presented in figures 2 and 3 clearly illustrate that the hydrolysate of fish collagen can be successfully used in whey beverages without causing changes in physico-chemical and sensory properties.

3.4. Antioxidant Activity of the beverages

Figure 4 shows that the freeze-dried feijoa puree and collagen hydrolysate contribute to the increase of the whey beverage total antioxidant activity. Addition of the minimum amount of collagen hydrolysate (0.25%) the activity was increased on 5-7%, all while the addition of the maximum amount of collagen hydrolysate (1%) contributed to the increase of the beverage total antioxidant activity on 12-15%.

4. Conclusion

Data of our researches have shown the potential of complex usage of dairy and fish wastes for production of functional beverages.

The rational dosage of fish collagen hydrolysate from salmon skin has been determined to be 0.5% of the total mass of whey beverage. The sensory has showed high gustatory qualities of the beverage with a pleasant faint hint of feijoa.

The technical process of obtaining the whey beverage with freeze-dried collagen hydrolysate from salmon skin and feijoa has been developed. It includes preparation of freeze-dried fish collagen hydrolysate, preparation of freeze-dried feijoa powder, restoration of dry cheese whey, addition of
ingredients into cheese whey according to the formulation, homogenization and pasteurization of the obtained beverage, cooling to 4±2 °C and holding at that temperature for 12 hours.

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