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An isotonic drink containing Pacific cod (*Gadus macrocephalus*) processing waste collagen hydrolysate for prevention of bone and cartilage disorders

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Abstract: Malnutrition is one of major factors of bone and cartilage disorders. Pacific cod (*Gadus macrocephalus*) processing waste is a cheap and highly promising source of bioactive substances, including collagen-derived peptides and amino acids, for bone and cartilage structure stabilization. Addition of these substances to a functional drink is one of the ways to achieve their fast intestinal absorption. Collagen hydrolysate was obtained via enzymatic hydrolysis, ultrafiltration, freeze-drying, and grinding to powder. The lyophilized hydrolysate was a light gray powder with high protein content (>90%), including collagen (about 85% of total protein) and a complete set of essential and non-essential amino acids. The hydrolysate was applicable as a protein food supply or a structure-forming food component due to presence of collagen fiber fragments. An isotonic fitness drink (osmolality 298.1 ± 2.1 mOsm/L) containing the hydrolysate and vitamin C as a co-factor in collagen biosynthesis was prepared. Addition of the hydrolysate did not adversely affect its organoleptic parameters. Production of such functional foods and drinks is one of the beneficial ways of fish processing waste utilization.

Keywords: bone and cartilage disorders; bioactive substances; Pacific cod; processing waste utilization; collagen hydrolysate; isotonic drink.

1. Introduction

Malnutrition is one of major factors of bone disorders. This has been reported for relatively healthy people with anorexia nervosa [1]. Risk of bone disorders is substantial in the cases of malnutrition for maintenance hemodialysis patients [2], kidney transplant recipients [3], and Parkinson's disease patients [4]. Various malnutrition-related cartilage disorders have also been reported, e.g. chronic inflammatory arthritis [5], or enlargement of cartilage [6].

Collagen is the main structural protein of bones, cartilage, ligaments, tendons in the joints. Hydrolyzed collagen, as a source of specific amino acids, can perform a building function and be a material for the formation of new collagen fibrils in the connective tissues of cartilage, thereby restoring them after damage [7]. Short collagen-derived peptides consisting of two or three amino acids are easily absorbed in the intestine [8]. If the collagen-derived peptides are consumed, the process of protein digestion in the gastrointestinal tract is facilitated [9], and the level of amino acids entering the cartilage increases; those amino acids are used further in collagen biosynthesis in chondrocytes [10]. From about 25 years of age, collagen biosynthesis slows down, and with age, the ratio of collagen types in tissues changes. Collagen becomes more rigid, which leads to a deterioration in the condition of all collagen-containing tissues of the human body. Therefore,

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maintaining of the musculoskeletal system performance is possible with the use of collagen derivatives in a daily dose of 10 g for 1–6 months [11].

Generally, only 30–50% of the fish wet weight is used in the production of fish fillets [12]. Fish processing waste is mostly used for fish meal or fish oil production, but it is a promising source of biologically active substances including collagen and its derivatives [13]. Pacific cod (*Gadus macrocephalus*) is one of the most captured fish species in Russia —427 600 tons were captured in 2021 [14]. Pacific cod processing waste actually comprises heads (21.5–25.6% of total wish wet weight); skin (5.2–6.0%); tails, swim bladders, spinal bones, clavicles, pectoral fins (2.8–14.8%); viscera: roe, milt, liver (16.3–23.9%) [15]. It has been reported that some peptides derived from Pacific cod bone via hydrolysis induced anti-osteoporosis effects in rats [16]. Pacific cod skin contains type I collagen which partial hydrolysate enhanced proliferation of fibroblast and osteoblast cells [17].

Addition of biologically active substances to functional drinks is one of the ways to achieve their fast intestinal absorption. The drinks may be supplemented with flavoring ingredients and enriched with functional components, including collagen hydrolysate for connective tissue regeneration [18,19]. Isotonic drinks are of great biomedical interest because they are developed for normalization of water and electrolyte content in the body and correction of imbalance of minor nutrients. When developing the formulation of an isotonic drink, the main attention is paid to the concentrations of carbohydrates and minerals contributing to the drink osmolarity that should be within 275–300 mOsm/L [20]. Vitamin C is known to be a cofactor in collagen biosynthesis [21]. Therefore, it is reasonable to combine collagen hydrolysate together with citrus fruits (lemon, orange, or grapefruit) with substantial vitamin C content (33.4–97.1 mg/100 g) or their juice concentrates [10,22].

Thus, Pacific cod processing waste may potentially be a cheap and highly promising source of bioactive substances for prevention of bone and cartilage disorders like osteoporosis. A vitamin C-containing isotonic drink is a promising basis of administration of such substances due to fast intestinal absorption, increased collagen biosynthesis rate and positive side-effects.

2. Materials and Methods

2.1. Collagen Hydrolysate Preparation

Samples of adult Pacific cod (*Gadus macrocephalus*) were obtained from local fish dealers in Khabarovsk region, Russia. The fish were caught by fishing companies in the northern part of the Sea of Okhotsk. The samples were dissected, and the tissues were mixed in a weight ratio typical for the cod processing waste [23]: heads with clavicles and muscle cutoffs—35%; skin with scales—22%; tails with tail fins—5%; spinal bones—20%; viscera (without roe, milt, liver)—18%. The mixture samples were washed with water at 8–12 °C for 20 min, kept frozen at –25 °C for 2 h and ground in a meat grinder with 3 mm hole grid.

Next, the mixture samples were hydrolyzed using *Bacillus licheniformis* protease with 50000 U/g proteolytic activity (“Protozyme”, manufactured by Biopreparat, Moscow, Russia). Fifty grams of tissue homogenate was mixed with 0.1 g of the enzyme and 50 mL of distilled water. The hydrolysis was performed in a thermostated water bath at 30 °C for 6 h. Next, the enzyme was inactivated by heating the mixture at 70 °C for 15 min, and the hydrolysate was separated from sediment by filtration through Whatman No. 3 filter paper.

The hydrolysate was mixed with 10% citric acid (ratio: 1:1) and treated for 4 h with constant stirring for odor neutralization. Next, the mixture was filtered in UF-401/402 ultrafiltration system (BioTechno Group, Moscow, Russia) using an aromatic polysulfonamide membrane filter with 100 kDa nominal molecular weight limit (Vladipor, Vla-

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dimir, Russia) at 50 °C. The filtrate was lyophilized in Lyopro-2.5 freeze dryer (Lyomac, Shanghai, China) in a plastic tray: freezing at –35 °C for 24 h and drying 30 °C for 5 h. The lyophilizate was finely ground in a mortar.

The whole procedure of hydrolysate preparation was performed in triplicate.

2.2. Water Content Determination

A sample was finely homogenized manually (with scissors and/or mortar). Water content was measured with MF-50 moisture analyzer (A&D, Tokyo, Japan). Each measurement was made in triplicate.

2.3. Total Protein Content and Total Nitrogen Content Determination

Total protein content was determined as 6.25 × total N content; the latter was determined using the Kjeldahl method with Kjeltec 1002 System Distilling Unit (Tecator, Höganäs, Sweden). Each measurement was made in triplicate.

2.4. Collagen Content or Total Content of Collagen-Derived Amino Acids Determination

Collagen content, or total content of collagen-derived amino acids (for collagen hydrolysate and drink samples), was determined as 7.4 × hydroxyproline content [13]. For hydroxyproline content determination, a homogenized sample (about 50 mg) was hydrolyzed by autoclaving in 1.0 mL of 6 M HCl in a sealed tube at 3.5 bar pressure for 3 h. One milliliter of 0.01 M $CuSO_4$, 1 mL of 2.5 M NaOH, and 1 mL of 6% H_2O_2 were added into the sample tube and a blank tube with 1 mL of distilled water. The solutions were mixed with occasional shaking for 5 min each time, and later placed in a water bath at 80 °C for 5 min with frequent intensive shaking. Next, the tubes were cooled in ice water, and 4 mL of 3.0 N (1.5 M) H_2SO_4 was added with shaking. Then, 2 mL of p-dimethylaminobenzaldehyde solution in n-propanol was added with thorough shaking. The tubes were heated at 70 °C for 16 min and cooled in tap water [24]. The absorbance at 540 nm of the prepared solutions was measured with PE-5300VI spectrophotometer (Ecroskhim, St. Petersburg, Russia). Hydroxyproline content was calculated using a previously made calibration with hydroxyproline standard (Sigma-Aldrich, St. Louis, MO, USA). Each measurement was made in triplicate.

2.5. Total Fat Content Determination

A sample was finely homogenized manually (with scissors and/or mortar). Total fat was extracted using SER 148 automated extraction system (VELP Scientifica, Usmate Velate, Italy). Total fat content was measured gravimetrically by weighing the extract. Each measurement was made in triplicate.

2.6. Ash Content Determination

A sample (about 5 g) was digested in a muffle furnace at 500–700°C for about 1 h to constant weight; the ash was collected and weighed. Each measurement was made in triplicate.

2.7. Powder Flow Determination

Powder flow was determined by measuring the time during a weighed portion of the hydrolysate passes (flows) through a funnel using a standardized method accepted in Russian pharmacopoeia [25]. A standardized funnel made of stainless steel with 110 mm upper inner diameter, 10 mm lower inner diameter and 40° vertical angle was used. A portion that would fill up 80–90% of the funnel was gained and weighed. The lower hole was plugged, the hydrolysate portion was poured through the upper hole, the lower hole was then unplugged, the time of portion passing was measured, and the powder flow was calculated in grams per second as portion weight / passing time. Each measurement was made in triplicate.

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2.8. Angle of Repose Determination

Angle of repose was determined as angle of dip of pile of hydrolysate formed after passing the funnel during powder flow determination (see Section 2.7) relative to horizontal plane. The angle was measured with a goniometer from three sides of the pile; the result was averaged. Powder flow rate was estimated using the standardized scale (Table 1). Each measurement was made in triplicate.

Table 1. Scale for powder flow rate estimation by angle of repose [25].

Powder flow rate	Angle of repose (°)
Very good	<30
Good	30–35
Satisfactory	35–45
Unsatisfactory	45–55
Bad	55–65
Very bad	>65

2.9. Enzymatic Activity Determination

The method is described in details in [26]. Briefly, the substrate solution of sodium caseinate standard (Sigma-Aldrich, St. Louis, MO, USA) was prepared by mixing 8 mL of 1 M NaOH, 36 g of urea, 10 mL of pre-prepared 22% sodium caseinate solution, 72 mL of distilled water, keeping that mixture at 25 °C for 30 min, and adding 10 mL of 1 M KH_2PO_4 and 4 g of urea. One milliliter of 2.35% hydrolysate suspension (concentration equal to its content in the drink) was added to 5 mL of the substrate solution; the mixture was stirred and kept at 25 °C for 10 min. Then 10 mL of 0.3 M trichloracetic acid was added, the mixture was stirred and filtered through Whatman No. 3 filter paper. Ten milliliters of 0.5 M NaOH was added to 5 mL of the filtrate, and 3 mL of phenol reagent [27] was rapidly added with stirring. The standard solution was prepared with adding 0.145 mg of tyrosine (Sigma-Aldrich, St. Louis, MO, USA) to 5 mL of 0.2 M HCl. Ten milliliters of 0.5 M NaOH was added to 5 mL of the mixture, and 3 mL of phenol reagent [27] was rapidly added with stirring. After 5 minutes, enzymatic activity was estimated by measuring tyrosine concentration using KFK-3-01 colorimeter (Zagorsk Optical-Mechanical Plant, Sergiyev Posad, Russia) with a red filter against the standard solution. Each measurement was made in triplicate.

2.10. Determination of pH

A SevenExcellence pH meter with InLab Expert Go-5m-ISM electrode (Mettler Toledo, Greifensee, Switzerland) was used to measure the pH value of the samples. Each measurement was made in triplicate.

2.11. Molecular Weight Analysis

Molecular weight distribution of 1% hydrolysate suspension filtered through Whatman No. 3 filter paper was analyzed using size-exclusion high-performance liquid chromatography with LC-10Avp chromatographer with SPD-10Avp UV/VIS Detector (Shimadzu, Columbia, MD, USA) equipped with TSKgel Alpha-M (30 cm × 7.8 mm, 13 μm particle size) and TSKgel Alpha-2500 (30 cm × 7.8 mm, 7 μm particle size) columns (Tosoh, Tokyo, Japan). 0.15 M NaCl solution was used as eluent with 0.8 mL/min elution speed. Absorbance was measured at 210 and 280 nm. The analysis was performed in triplicate.

2.12. Amino Acid Analysis

Amino acid concentrations in the samples of hydrolysate were measured using chromatographic analysis of their orthophtalic derivatives according to standard amino

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acid samples. First, 10 mg of a sample was resuspended in 1 mL of distilled water. The resulting suspension was 25× diluted, and 50 µL of the diluted suspension was dried up in an ampoule. Then, 100 µL of 6 M HCl was added to it and the ampule was sealed under vacuum. Acidic hydrolysis was performed over 24 hours and at 110 °C. After that, the ampoule was opened and the solution was dried up in the Eppendorf 5301 vacuum concentrator (Eppendorf, Hamburg, Germany). Finally, 50 µL of 0.1 M HCl was added to the dried sediment. The chromatographic separation was performed using Agilent 1200 series chromatographic system equipped with fluorescent detector and ZORBAX Eclipse AAA (15 cm x 4.6 mm; 5µm particle size) column (Agilent, Santa Clara, CA, USA). The mobile phases were mixtures of 40 mM pH 7.8 phosphate buffer (Solution A) and 80% water solution of acetonitrile (Solution B). Borate buffer with pH 10.2 and o-phtalaldehyde were used for amino acid derivatization. The amino acid derivatives were eluted at flow rate of 1 mL/min with a gradient of the Solution B. Total run time was 41 min including 3 min of flushing with 63% Solution B and 2 min of re-equilibration to 2% Solution B. The areas under the fluorescent chromatogram peaks of the analyzed sample and of the amino acid standards (Agilent, Santa Clara, CA, USA) were measured. Some other details of the procedure are described in [28]. The analysis was performed in triplicate.

2.13. Isotonic Drink Preparation

The samples of isotonic drink were prepared according to their formulations presented in Table 2. The fruit juice concentrates were prepared from natural fruits purchased in food stores in Moscow, Russia. The fruits were washed with hot tap water; next, their pulp was separated from rind, juice was extracted from pulp with a manual squeezer and filtered through four layers of gauze. The components were mixed in Turbula 2.0 laboratory mixer (Vibrotechnik, St. Petersburg, Russia) for 10 min at 50 min^{-1} rotation speed. The procedure of isotonic drink preparation was performed in triplicate.

Table 2. Isotonic drink formulations. The purchased components had food quality grade.

Component	Manufacturer	Content (% by weight)		
		Lemon drink	Orange drink	Grapefruit drink
Drinking water	Svyatoy Istochnik, Kostroma, Russia	75.14	78.89	76.07
Fruit juice concentrate	Authors; extracted from natural fruits	18.78	15.03	17.85
Fructose	Molecularmeal, Moscow, Russia	2.09	2.09	2.09
Glucose	Molecularmeal, Moscow, Russia	1.17	1.17	1.17
Collagen hydrolysate	Authors	2.35	2.35	2.35
Sea salt	Mareman, Tashkent, Uzbekistan	0.47	0.47	0.47

2.14. Osmolarity Determination

Osmolarity of the drink samples was measured with Osmo Station OM-6060 automatic freezing point depression osmometer (ARKRAY, Kyoto, Japan). Each measurement was made in triplicate.

2.15. NaCl Content Determination

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Eighty milliliters of a drink sample was mixed with 1 mL of 0.25 M $K_4[Fe(CN)_6]$, then 1 mL of 1 M $Zn(CH_3COO)_2$ dissolved in 3% CH_3COOH was added, the mixture was vigorously shaken, and the volume was adjusted to 100 mL by distilled water (Solution 1).

Five hundred grams of preliminarily ground $NH_4Fe(SO_4)_2 \cdot 12H_2O$ was dissolved in 1 L of boiling distilled water. Then, the solution was cooled with cold tap water and filtered through Whatman No. 3 filter paper. Concentrated HNO_3 was added to the filtrate by small portions until the solution became transparent (Solution 2).

Titration burette was fulfilled with 0.1 M KSCN. Another titration burette was fulfilled with 0.1 M $AgNO_3$. Five milliliters of concentrated HNO_3, 2 mL of Solution 2 and 2 drops of 0.1 M KSCN from the burette were added to Solution 1, and the mixture was shaken. Next, 0.1 M $AgNO_3$ had been added from the burette with constant shaking until red color disappeared, with a little excess. Then, 0.1 M KSCN from the first burette had been added until stable within 30 s red-brown color appeared.

The same procedure was performed with 80 mL of distilled water instead of a drink as a blank test. NaCl content was calculated in g/L as 0.073 × ((volume of 0.1 M $AgNO_3$ expended for drink sample (mL) – volume of 0.1 M $AgNO_3$ expended for blank sample (mL)) – (volume of 0.1 M KSCN expended for drink sample (mL) – volume of 0.1 M KSCN expended for blank sample (mL))). Each measurement was made in triplicate.

2.16. Total Carbohydrates Content Determination

The content of total carbohydrates was determined using BioLC Dionex high-performance ion chromatographer equipped with CarboroPac PA 1 (25 cm × 4 mm, 10 μm particle size) column (Thermo Fisher Scientific, Waltham, MA, USA). HPLC grade distilled water was used as eluent with 1.0 mL/min elution speed. Content of each carbohydrate was measured by peak area calibrated with solutions of standards of all detected carbohydrates (standards manufactured by Sigma-Aldrich, St. Louis, MO, USA). The content of total carbohydrates was calculated as a sum of content of all detected carbohydrates. Each measurement was made in triplicate.

2.17. Vitamin C Content Determination

The method is described in details in [29]. Briefly, vitamin C was determined using Milichrom A-02 high-performance liquid chromatographer equipped with Prontosil 120-5 C18 (7.5 cm × 2 mm, 5 μm particle size) column (EcoNova, Novosibirsk, Russia). A gradient of 0.4 M $LiClO_4$ (pH 2.4) and acetonitrile was used as mobile phase. Vitamin C was detected at 2.5 min retention time and quantified by measuring the absorbance at 240 nm. Each measurement was made in triplicate.

2.18. Viscosity Determination

Viscosity of the drink samples was measured with Polymer RPE-1M rotational viscosimeter equipped with T1-B1 cylinder system (Khimpribor-1, Tula, Russia). Each measurement was made in triplicate.

2.19. Statistical Analysis

The numerical data are presented as means ± SD or lower value–upper value. Significance of the difference between groups of samples being compared (Table 3) was evaluated using Mann–Whitney *U* test; the level of significance considered was $p < 0.05$. Data analysis was performed using STATISTICA 9.0 software.

3. Results and Discussion

3.1. Chemical Composition of Pacific Cod Processing Waste Components

Chemical composition of waste components is presented in Table 3. The difference between the components by collagen and protein content was not substantial, except for

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heads with clavicles and muscle cutoffs and tails with tail fins. Most likely, the deficiency of collagen in those components is caused by presence of collagen-free muscle. The total fat content of the samples is suitable for subsequent freeze-drying; samples rich in lipids are not recommended for freeze-drying due to low quality of lyophilizate [30].

Table 3. Chemical composition of Pacific cod processing waste components. Values are expressed as % by wet weight (means ± SD; n = 3). Different superscripts in each column indicate significant difference (Mann–Whitney *U* test: $p < 0.05$).

Component of waste	Water content	Total protein content	Collagen content	Total fat content	Ash content
Heads with clavicles and muscle cutoffs	78.21 ± 1.43[a]	15.71 ± 0.39[a]	10.02 ± 0.24[a]	0.77 ± 0.01[a]	5.37 ± 0.14[a]
Skin with scales	75.79 ± 1.39[b]	18.61 ± 0.46[b]	14.38 ± 0.34[b]	1.03 ± 0.02[b]	3.56 ± 0.08[b]
Tails with tail fins	79.55 ± 1.45[a]	14.38 ± 0.36[a]	11.52 ± 0.27[a]	0.53 ± 0.01[c]	5.54 ± 0.14[a]
Spinal bones	74.32 ± 1.36[b]	18.25 ± 0.45[b]	13.51 ± 0.32[b]	0.75 ± 0.01[a]	6.68 ± 0.15[c]
Viscera (without roe, milt, liver)	74.47 ± 1.36[b]	19.96 ± 1.36[b]	14.98 ± 0.35[b]	2.58 ± 0.06[d]	2.99 ± 0.06[b]

3.2. Quality and Chemical Parameters of Collagen Hydrolysate

Some values of quality and chemical parameters of the lyophilized collagen hydrolysate are summarized in Table 4. The lyophilizate was formed as porous plates with the width of 3–6 mm, light gray color, insipid odor and flavor, and brittle structure. The plates were subsequently ground to powder which had satisfactory flow rate according to the value of angle of repose. The powder was partly soluble, but the insoluble fraction was negligible for the purpose of a functional drink preparation. The powder had none enzymatic activity and lightly acidic nature. The quality parameters of the hydrolysate make it acceptable as a food or drink component [31].

Table 4. Quality and chemical parameters of lyophilized collagen hydrolysate. Numerical values are expressed as lower value–upper value; n = 3.

Parameter	Value
Appearance before grinding	Porous plates
Appearance after grinding	Homogenous finely dispersed powder
Odor	Insipid
Flavor	Insipid
Color	Light gray
Powder flow (g/s)	0.83–0.91
Angle of repose (°)	42–45
Solubility in water	Partial
Enzymatic activity	None
pH of 10% water suspension	4.5–4.9
Molecular weight (kDa)	10.6–110.8
Water content (% by weight)	4.2–6.5
Total protein content (% by weight)	90.6–93.5
Total content of collagen-derived amino acids (% by total content of amino acids)	84.3–86.1
Total fat content (% by weight)	0.6–0.9

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Ash content (% by weight)	1.7–2.0

Molecular weight distribution of the collagen hydrolysate being studied was typical for fish collagen enzymatic hydrolysate [13,32]. High content of amino acids makes the hydrolysate applicable as a protein food supply and a structure-forming food component due to presence of collagen fiber fragments. Collagen hydrolysate had relatively high content of glycine, proline and hydroxyproline (about 50% in total), which is typical for untreated fish collagen [26] (see Table 5). Trace content of tryptophan was mostly derived from fish muscle protein of muscle cutoffs [33,34]. Anyway, the hydrolysate contains the complete set of essential and non-essential amino acids and is applicable as a protein supply. Low lipid content (<1%) makes the hydrolysate applicable as a component of food or drinks for low-calorie and very low-calorie diets [35].

Table 5. Amino acid composition of lyophilized collagen hydrolysate. Content values are expressed as lower value–upper value; n = 3.

Amino acid	Content (% by weight)
Lysine	4.12–4.34
Histidine	1.46–1.51
Arginine	4.51–4.65
Aspartic acid	5.61–5.79
Threonine	2.29–2.36
Serine	2.83–2.92
Glutamic acid	9.98–10.25
Proline	9.20–9.44
Hydroxyproline	7.71–7.96
Tryptophan	0.10–0.12
Cysteine	0.12–0.14
Glycine	28.08–28.94
Alanine	4.97–5.13
Valine	2.06–2.13
Methionine	0.04–0.05
Isoleucine	2.23–2.30
Leucine	3.21–3.31
Tyrosine	0.03–0.04
Phenylalanine	1.28–1.32
Hydroxylysine	0.77–0.80

3.3. Isotonic Drink Properties

Applicability of the Pacific cod processing waste collagen hydrolysate as a component of isotonic fitness fruit drinks has been studied. The drink formulations (see Table 2) were designed to make them isotonic (osmolarity 275–300 mOsm/L). The mineral electrolytes and low-molecular carbohydrates should be included into a fitness drink; their content should be adjusted to achieve the necessary osmolarity level. Addition of wholesome biologically active substances (e.g. proteins, amino acids, vitamins) is acceptable [20]. In the case of our collagen hydrolysate-containing drink, the necessary osmolarity was achieved by adjusting concentrations of salt, carbohydrates and juice concentrates. The citrus fruit juices are also sources of vitamin C known as a cofactor in

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collagen biosynthesis [21]. Collagen hydrolysate was added to the drink as another bioactive component for bone and cartilage structure stabilization. As mentioned above, it contains substantial content of proline and hydroxyproline. These two amino acids are crucial for collagen triple helix stability due to formation of extra hydrogen bonds [36,37]. Proline and hydroxyproline are both non-essential (or conditionally essential) amino acids, but their biosynthesis rate may not cover the requirement of collagen biosynthesis, therefore they should be additionally consumed for prevention of bone and cartilage disorders [38].

Some physicochemical and organoleptic parameters of the drink prepared in three flavor variations according to the designed formulations are presented in Table 6. The drink was slightly acidic, which is typical for citrus fruit drinks. The content of hydroxyproline and total collagen-derived amino acids characterizes the drink as an additional source of bioactive substances for collagen biosynthesis. So does the content of vitamin C; administration of 500 mL of the drink (in any variation) covers >40% of the recommended daily dose of vitamin C in the United States and Canada [39]. Organoleptic estimation of the drink showed that its odor, flavor and color were typical for any drink of the respective flavor variation (lemon, orange, or grapefruit) without any influence of collagen hydrolysate. Despite partial water solubility of the collagen hydrolysate, the finished drink had no solid matter visible to the naked eye.

Table 6. Physicochemical and organoleptic parameters of isotonic drink containing lyophilized collagen hydrolysate. Numerical values are expressed as means ± SD; n = 3.

Parameter	Lemon drink	Orange drink	Grapefruit drink
Osmolarity (mOsm/L)	299.1 ± 1.1	297.5 ± 1.6	298.3 ± 1.8
NaCl content (g/L)	5.7 ± 0.4	6.1 ± 0.3	5.8 ± 0.4
Total carbohydrates content (g/L)	46.5 ± 3.6	42.3 ± 3.4	44.1 ± 2.3
Total nitrogen content (g/L)	3.9 ± 0.3	3.3 ± 0.2	3.6 ± 0.3
Total collagen-derived amino acids content (g/L)	19.9 ± 1.2	18.0 ± 1.0	20.5 ± 1.4
Vitamin C content (mg/L)	75.5 ± 8.9	90.4 ± 10.4	72.4 ± 9.3
pH	4.5 ± 0.1	4.8 ± 0.1	4.6 ± 0.0
Viscosity (kPa·s)	1.14 ± 0.23	1.16 ± 0.19	1.19 ± 0.24
Color	Light yellow	Yellow	Light red
Odor	Lemon-like	Orange-like	Grapefruit-like
Flavor	Sour-sweet	Sour-sweet	Sour-sweet
Transparency	Homogeneously turbid	Homogeneously turbid	Homogeneously turbid

4. Conclusions

Pacific cod processing waste is a mass-production waste; therefore, it is a promising source of low-cost collagen and other bioactive substances. The hydrolysate of collagen obtained from Pacific cod processing waste is rich in amino acids, especially proline and hydroxyproline which are necessary for collagen biosynthesis. The quality parameters of the hydrolysate make it acceptable as a protein or structure-forming component of food or drinks, including food or drinks for low-calorie and very low-calorie diets. Addition of protein hydrolysate to a functional drink is one of the ways to achieve fast intestinal absorption of its components. The hydrolysate-containing isotonic fitness drink had acceptable physicochemical and organoleptic parameters and substantial content of vitamin C known as a cofactor in collagen biosynthesis. Therefore, the designed functional drink is a promising source of bioactive substances for prevention of bone and cartilage

disorders. Mass manufacturing of such drinks is one of the beneficial ways of fish processing waste utilization.

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