Agronomy Research **17**(S2), 1287–1298, 2019
https://doi.org/10.15159/AR.19.097

Use of lipids of Chlorella microalgae in poultry meat marinades and sauces recipes

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Abstract. The aim of this study is to develop formulations and technologies for fermented poultry meat products with the addition of whey and lipid extracts obtained from Chlorella microalgae. Lyophilized microalgal biomass was obtained from cell suspensions of *Chlorella sorokiniana* (strain 211-8k) cultivated in a closed photobioreactor under laboratory conditions. For the cell wall disintegration, the biomass samples were homogenized using a high-speed homogenizer at 10,000 vol min^{-1} for 5 minutes. The lipid extraction was performed on a Sohxlet apparatus Buchi E-812 SOX with the solvent extraction system ethanol: n-hexane (1: 9). The higher fatty acids composition of the obtained microalgal lipid extracts was determined by gas chromatography with flame-ionization detection using nitrogen as a carrier gas. The ω-3 and ω-6 content represented 26.59% and 19.05% respectively, which indicates that these lipid extracts have high nutritional values. The curd whey was obtained from cow's milk of summer and winter production from 2017 to 2018 (Lomonosov district auxiliary farm, Leningrad region); and lyophilized Direct Vat Set (DVS) cultures (Ch. Hansen, Denmark). The organic acids and carbohydrate content in the serum was determined by ion-exclusion HPLC. The FD-DVS CHN-19 culture was selected to produce a serum with improved organoleptic characteristics and a lower propionic acid content (0.01 g L^{-1}). To obtain an optimal ω-3 / ω-6 ratio, a phyto-additive mixture based on sunflower oil and lipid extracts from *C. sorokiniana* microalgae at a ratio of 5–10: 1 is proposed to be used in recipes and technologies of sauces and marinades. It is established that the use of curd whey marinades allow to increase the water-holding capacity (WHC) by 6−8% and to reduce losses during heat treatment of poultry meat from 2 to 11%.

Key words: microalgal biomass, *Chlorella*, microalgal lipid extract, curd whey, DVS-cultures, marinades and sauces, poultry meat.

INTRODUCTION

Building a national system for the production of healthy food using innovative food ingredients is one of the priorities of the Russian technological platform BIOTECH 2030.

Analysis of the current nutrition and assessment of the nutritional status of the Russian population in various regions shows, that for most population categories, diet is significantly deficient in omega-3 and omega-6 polyunsaturated fatty acids and a wide range of vitamin-like substances of natural origin (Hu et al., 2008). In this regard, it is highly important to develop and improve Eco-friendly production technologies of biologically active compounds.

Microalgae are rich sources of valuable nutrients, antioxidants and cancer-preventive compounds (Reyna-Martinez et al., 2018). Chlorella miroalga is a promising genus as its biomass is characterized by a high specific growth rate and a rich protein, carbohydrate, essential fats and pigment content.

Microalga *Chlorella sorokiniana* has a high growth with a maximum specific growth rate that can reach 0.12 h^{-1} and a productivity of 0.66 g L^{-1} (Lizzul et al., 2018). The biomass contains in average 40%, carbohydrates - 30–38%, lipids - 18–22% by dry weight (Belkoura et al., 1997; Lizzul et al., 2018). Microalgae biomass is a source of antioxidants such as carotenoids (Damerg et al., 2017) that can reach 0.69% of the dry weight (Lizzul et al., 2018). A previous study compared four microalgae species encluding Chlorella pyrosenoida. The average PUFA content reached 61.17% and chlorella biomass was found to have a high palmitic acid (59.12%) and oleic acid (29.69%) content (Silva Gorgônio et al., 2013).

It is necessary to adapt and improve the cultivation and deep processing of microalgae biomass with a high added value before reaching the stage of a large-scale production (Matos, 2017).

The issue of whey processing in northwest Russia is relevant and has been long discussed. 50% of the dry matter of milk passes to the whey. This includes 20% of the total proteins, 95% of the lactose, 80% of the mineral substances and 10% of milk fat by dry weight. Also, whey has the ability to loosen muscle proteins and emulsify fat.

The development of food formulations such as emulsions stabilized by whey with the addition of microalgae lipids is fairly relevant. The serum obtained after the acid coagulation of milk contains a wide range of Lactobacillus. These bacteria secrete lactic acid, use carbohydrates as an energy source (Hansen, 2002; Leroy & De Vuyst, 2004; Seskin & Bazarnova, 2016) and are widely used in the meat fermentation technology.

However, the specific flavor and short shelf life of whey create problems for its widespread use in food formulations. The organoleptic characteristics of dairy products largely depend on the hydrolysis of milk protein and fat resulting in the accumulation of nitrogenous compounds, free amino acids and fatty acids, which are precursors of many taste and aroma-forming substances (Korenman et al., 2006; Boeva, 2007). The formation of these compounds depends highly on the enzymatic activity of lactic acid bacteria (Evans et al., 2009; Croissant et al., 2011). The components responsible for most of the peculiar smell of cottage cheese whey are volatile fatty acids: acetic, propionic, butyric and formic acids. Reducing the specific taste and smell of whey is possible by selecting the appropriate cultures of microorganisms for milk fermentation and by masking the taste with flavoring additives. Actually, the use starter culture so-called DVS culture - Direct Vat Set meaning ‘direct introduction of pure cultures into processed milk’, is quite popular for the production of fermented milk products. The DVS cultures include strains of Lactococcus lactis subspecies cremoris, Lactococcus lactis subspecies lactis, Leuconostoc mesenteroides subspecies cremoris and Lactococcus lactis subspecies diacetylactis (Varnam & Sutherland, 1994; Kuznetsova, 2005). To obtain dairy products and whey with predictable properties, it is advisable to preliminarily study the activity of the fermentation enzymes, composition of which differs in the ratio of strains that the manufacturer does not report.

Broiler chicken meat includes high-grade, highly digestible animal proteins but has a low content of essential lipids (Donskova et al., 2018). It would be valuable to develop recipes for seasoning chicken meat semi-finished products and other food products that will be enriched with essential fatty acids.

The peculiar development of the sauces market is related to higher incomes and an increase in travelling opportunities. Consumers are increasingly inclined to experiment with food and get more interested in world cuisine. A promising direction for the development of sauces is the creation of seasoning dishes based on natural food additives, without the use of synthetic preservatives, flavors, antioxidants and dyes (Böhm et al., 1998). The seasoning sauces category is the most promising niche for innovation, packaging experiments and flavors.

The aim of this study is to develop formulations and technologies for marinades and sauces for poultry meat using whey additioned with lipid extracts from *Chlorella* microalgae.

MATERIALS AND METHODS

This study was carried out in the Graduate School of Biotechnology and Food Science and in the LTC 'Analytics. Materials. Technologies 'SPbPU.''

To obtain microalgae biomass lyophilisates, crude cell suspensions of *C. sorokiniana* (strain 211-8k) from the collection of algae at the University of Göttingen (international Acronym SAG) were cultivated under laboratory conditions. The biomass cultivation took place in a pilot bioreactor (Fig. 1).

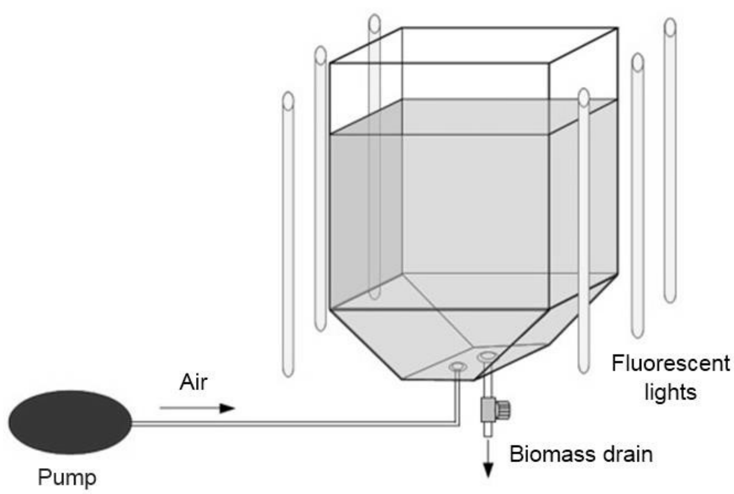

Figure 1. Cultivation of *Chlorella sorokiniana* microalgae in a pilot bioreactor.

For a surface illumination regulated to a day-night mode, a fluorescent lamp was used at luminous flux of 2,500 ± 300 Lx, T (K) 400. The temperature of cultivation was 23 ± 1 °C; the intensity of aeration of the mixture - 1.5 L min^{-1}; the mixing mode - periodic (15 min once a day) and the mixing speed - 500 rpm. The used cultivation medium is described as balanced in macro - and microelements (Crofcheck et al., 2012). The maximum concentration 41×10^6 cells mL^{-1} was reached on the 9th day of cultivation. The specific growth rate of the linear phase of biomass growth was $\mu = 0.26$ day^{-1} (Politaeva et al., 2017).

The concentration of the biomass was performed by auto-flocculation. In the depletion phase, a 0.1 N NaOH solution was added during cultivation while constantly stirring. At pH 11, the flocculation efficiency reached 95.4% and the flocculation took no more than 30 minutes (Bazarnova et al., 2018; Kuznetsova et al., 2018).

The microalgae suspensions concentrates were lyophilized under 1 mbar at a temperature of -55 °C in an Alpha 1–2 LD plus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH).

For a mechanic cell wall disintegration, a 3 g of freeze-dried biomass sample was mixed with 10 mL of mixture of n-hexane: ethanol (9:1) and subjected to homogenization using a Silent Crusher M high-speed homogenizer (IKA® Werke, T25 Basic) at 10,000 vol min^{-1} for 5 minutes. The lipid extractions were performed on a Sohxlet apparatus Buchi E-812 SOX with the solvent extraction system ethanol: n-hexane (1: 9) for 15 cycles and the extraction lasted 2 hours in total. These parameters were shown to be optimal for a maximum lipid yield in a previous study (Toumi et al., 2018). The extraction temperature corresponded to the boiling point of the solvent mixture, which was 69–70 °C.

The higher fatty acids composition in the lipid extract of microalgae *C. sorokiniana* was determined by gas chromatography with flame-ionization detection Agilent Technologies Sales & Services GmbH & Co.KG, on a BPX70 column (60 m × 0.25 mm × 0.25 micron), SGE Analytical Science, VWR International GmbH; using nitrogen as a carrier gas. For their identification, the Supelco 37 Component FAME Mix standard containing 37 fatty acid methyl esters was used.

Curd whey was obtained from cow's milk of summer and winter production from 2017 to 2018 (Lomonosov district auxiliary farm, Leningrad region). This serum was produced by acid coagulation of cow's milk with a 3.5% fat by mass using lyophilized DVS cultures for cottage cheese and cheese making (Ch. Hansen, Denmark). To establish the compliance of the DVS starters culture with the specified microflora composition, they were pre-microscoped after activation by adding them to pasteurized skimmed milk and thermostating at 38 °C for 24 hours.

The proteolytic activity of the starter's microflora was determined by the method of Anson and Mirsky (Srichanun, 2012). The organic acids and carbohydrate content in the serum was determined by ion-exclusion HPLC according to (Kruchina-Bogdanov & Anosov, 2003). The chromatographic system included a Water 510 pump, a Rheodyne sample injector (volume 20 µl) and a 9 × 600 mm glass column with resin Hitachi-2614. A column with a water jacket allowed the separation of a series of organic acids contained in the samples. A 90 mM sulfuric acid in 2% by volume of aqueous acetonitrile as used as a mobile phase; with an eluent feed rate of 1.4 mL min^{-1} at a column temperature of 50 °C. All reagents were analytical grade.

The fatty acid composition of whey was determined by Gas Chromatography (GC) according to on a CBP 5-25 column (25 m × 0.22 mm × 0.2 µm), carrier gas – nitrogen at a rate of 20 cm s^{-1}; flame ionization detector; temperature gradient - from 70 to 300 at a rate of 4 °C min^{-1} (Deeth et al., 1983; Liguori et al., 2015). The temperatures of the injector and the detector were 280 °C and 311 °C in this order. Tridecanoic acid (REAHIM AO LLC) was used as an internal standard.

Marinades and sauces were prepared from pasteurized whey (95 °CC, 20–30 min) and refined sunflower oil with the addition of lipid extracts of *C. sorokiniana* microalgae.

Marinated semi-finished products from poultry meat were produced from chilled broiler chicken carcasses produced by the Udarnik poultry farm (Leningrad Region). Marinating broiler chicken meat was carried out at a temperature of 2–4 °C for 12 hours. The functional and technological parameters of meat were examined before and after marinating: the moisture content and water-holding capacity (WHC) of meat, as well as losses during heat treatment (roasting).

RESULTS AND DISCUSSION

The main technological steps for microalgae lipid extraction included the preparation of cell suspension, dehydration of biomass, mechanical disintegration of the cell wall, extraction in Sohxlet apparatus and obtaining lipid extract after evaporating the extraction solvents (Fig. 2).

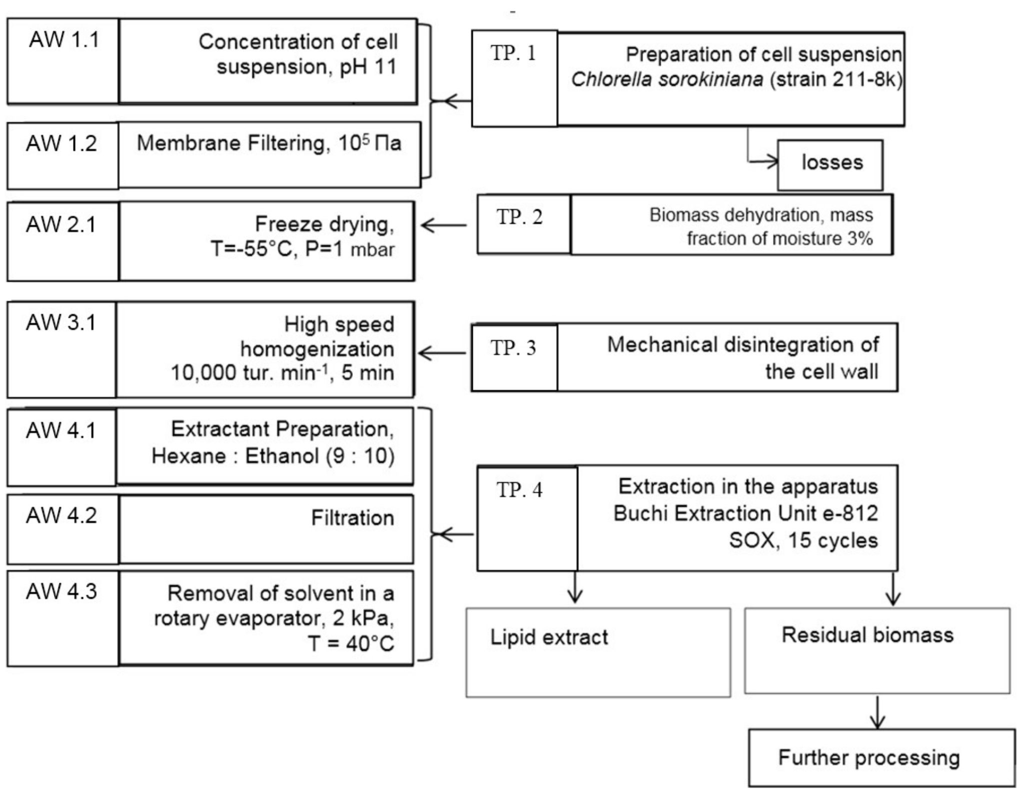

Figure 2. Scheme of the leading steps to obtaining lipid extracts from *C. sorokiniana* biomass. TP - technological process, AW - auxiliary work.

The lipid yield at the selected extraction mode represented about 20% by dry weight of microalgal biomass, which corresponds to the previously obtained results (Silva Gorgônio et al., 2013; Lizzul et al., 2018). The obtained lipid extracts are characterized by a dark brown color and a pronounced fishy taste and smell, therefore it was proposed to include these extracts to edible vegetable oils for further use in oil-based spreads, sauces and marinades.

The fatty acid composition of the lipid extract obtained from lyophilized biomass samples of *C. sorokiniana* microalgae is shown in Table 1.

The ω-3 and ω-6 fatty acid content in the lipid extracts as well as the FAO / WHO recommendations on the optimal content of saturated, monounsaturated and polyunsaturated fatty acids are listed in Table 2.

Among all the identified fatty acids, palmitic acid, trans and cis-linoleic acids, as well as α-linolenic acid, have the highest yield, which corresponds to the data on the study of high-fatty acids in several species freshwater algae. An increase in the oleic acid content (3,670 mg per 100 g of dry biomass according to our results) is associated with adaptive effects in response to stress factors or an increased duration of cultivation (Molino et al., 2018; Van Wagenen et al., 2012).

Table 1. Fatty acids composition of the lipid extracts of *C. sorokiniana*

Yields, %	Cn:m	Fatty acids
17.80	16:0	palmitic acid
0.80	16:1	palmitoleic acid
1.88	17:1	cis-10-Heptadecenoic acid
1.72	18:0	stearic acid
18.35	18:1, 9	oleic acid
6.10	18:1, 7	vaccenic acid
3.32	18:2, 6	trans-linoleic acid
14.80	18:2, 6	cis-linoleic acid
7.54	18:3, 6	octadecatriene acid
26.06	18:3, 3	α-linolenic acid
0.93	20:4, 6	α-linolenic acid
0.53	20:5, 3	eicosapentaenoic acid
0.17	23:0	tricosanoic acid

Table 2. Characterization of the biological value of *C. sorokiniana* lipid extracts

Lipid fractions	Content in microalgal lipids, %	FAO/WHO recommendations, g 100 g^{-1} of lipids*
Unsaturated Fatty Acids (UFA)	19.7	30.0
Monounsaturated Fatty Acids (MUFA)	27.1	60.0
Polyunsaturated Fatty Acids (PUFA)	53.2	10.0
total *ω-3*	26.6	
total *ω-6*	19.1	

*– FAO. 2010. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO 'Food and Nutrition Organisation'. Geneva, 91. Rome.

The ratio of saturated to unsaturated fatty acids in the lipid extract represented 3:10, and the fatty acids: PUFA ratio constituted 8:10. The high ω-3 and ω-6 content indicates a high nutritional value of the selected lipids (Meyer et al., 2003, Silva Gorgônio, Gomes Aranda & Couri, 2013). However, a fat with high content of essential unsaturated fatty acids does not guarantee its usefulness and indicates a need to assess their balance and redundancy.

For the use of microalgae lipids in the formulation of emulsion products, it is advisable to prepare concentrates in edible vegetable oils, which can be mixed with the fatty basis of spreads, sauces, marinades. As previously stated, the lipid extract is brown and has a pronounced fish like smell, so, it is advisable to select its dilution in such a way as to exclude foreign flavor in food products.

The World Health Organization and a number of national medical organizations have recommended a daily intake of 500–1,000 mg of ω-3 for the prevention of cardiovascular diseases (Simopoulos, 2002; Kris-Etherton et al., 2009). At the same time, the ratio of the consumed ω-6 and ω-3 polyunsaturated fatty acids should be no higher than 2: 1-3:1 according to the recommendations of the US National Institutes of Health (Davis, Kris-Etherton, 2003).

The microscopy of the starter cultures used for fermentation of milk showed no sign of extraneous microflora (yeast, mycelium of microscopic fungi). In the CHN-11 sample, short chains of streptococci predominated (4–5 pieces in the chain). Diplococci and cocci were present as well. Sample CHN-22 showed similar results. The CHN-19 sample showed a distinctive feature: predominance of long chains of cocci in the form of beads with 8–10 cocci per chain in average. The most intensive development of cocci was observed after 6 hours of fermentation. During the depletion phase, 24 hours after the start of ripening, their proportion decreases, which is associated with the adverse effect of acid accumulated as a result of fermentation. The study of the dynamics of acid accumulation during milk fermentation indicated that, in all three samples of the activated starter cultures, the process proceeded almost equally.

The results of the study of the proteolytic activity of the starter microflora under different acidity values of the medium showed that, at pH 5, the proteolytic activity of the CHN-19 starter was significantly higher than the in CHN-11 and CHN-22 samples.

The results of organic acids and carbohydrate composition of milk whey are presented in Table 3 and Fig. 3 shows their chromatographic profiles.

Table 3. The organic acids and carbohydrate composition of milk whey

	Content, g L^{-1}		
Organic acids	CHN-11	CHN-22	CHN-19
citric acid ($C_6H_8O_7$)	0.07 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
malic acid ($C_4H_6O_5$)	1.04 ± 0.05	1.01 ± 0.05	0.71 ± 0.04
pyruvic acid ($C_3H_4O_3$)	0.25 ± 0.01	0.45 ± 0.02	0.04 ± 0.01
succinic acid ($C_4H_6O_4$)	0.24 ± 0.01	0.32 ± 0.02	0.24 ± 0.01
lactic acid ($C_3H_6O_3$)	5.07 ± 0.25	5.24 ± 0.26	5.57 ± 0.29
acetic acid (CH_3COOH)	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
propionic acid ($C_3H_6O_2$)	0.06 ± 0.01	0.09 ± 0.01	0.010 ± 0.002
butyric acid ($C_4H_8O_2$)	0.050 ± 0.003	0.051 ± 0.003	0.042 ± 0.003
lactose ($C_{12}H_{22}O_{11}$)	16.08 ± 1.10	17.07 ± 0.85	17.51 ± 0.87
galactose ($C_6H_{12}O_6$)	6.86 ± 0.35	6.67 ± 0.33	5.07 ± 0.25

The highest lactic acid content in whey was reached using the CHN-19 starter culture. This serum distinguished itself from others as it had a higher proteolytic activity and better organoleptic indicators due to a decrease in propionic and butyric acid content, giving it a 'whey' flavor (Korenman, 2006). It was established that the best organoleptic indicators characterized the serum obtained by fermenting milk with CHN-19 cultures.

Marinade formulations based on curd whey and refined sunflower oil additioned with *C. sorokiniana* lipids were developed. Serum content in the marinades ranged from 40 to 50%; sunflower oil with phytoadditives - from 15 to 30%; dry mixes of spices - from 2.5 to 2.7% by dry weight of marinades.

To get the necessary consistency for the marinade, a mixture of guar and xanthan gums was used at a ratio of 1: 7, the content of which represented 1.5% by weight of marinade.

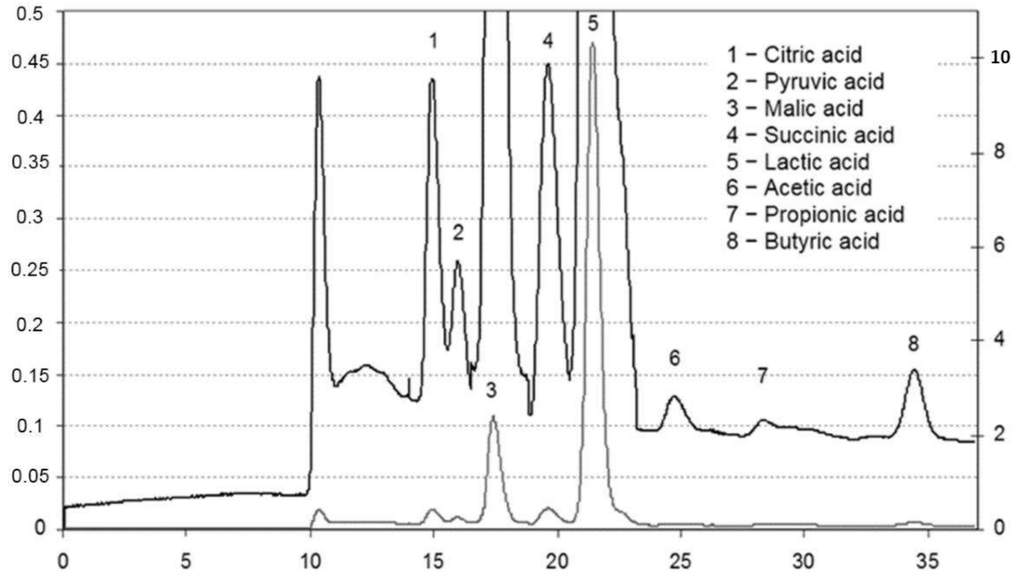

Figure 3. Chromatographic profile of organic acid in milk whey. X axis – time (h); Y axis – optical density at 210 nm.

The preparation of the marinade included the preparation of an emulsion base, for which the required amount of refined sunflower oil with the addition of microalgae lipids was loaded into the mixer at a temperature of 3–5 °C, water and curd serum at a temperature of 3–5 °C and mixed for 1 minute until an emulsion was formed, then the remaining water was added and the dry components of the marinade were evenly spread over the entire surface and mixed for 7–10 minutes until a homogeneous mass was obtained.

Ground spices, spicy greens, and whole spice seeds were used to compile the aromatic composition of marinades. Spice mixtures were added to the finished emulsion.

Table 4. Functional and technological indicators of marinated chicken meat

	Functional and technological indicators of chicken meat			
Whey content in marinades, %	Humidity content, %	pH	WHC, %	Losses after heat treatment, %
Breast fillet raw material				
-	76.9 ± 0.5	6.3	47.6 ± 0.3	30.1 ± 0.2
Marinated breast fillet				
Control	75.1 ± 0.5	6.5	53.3±0.3	38.3 ± 0.2
30	77.0 ± 0.5	6.4	54.9 ± 0.3	36.1 ± 0.2
50	78.5 ± 0.5	6.2	55.3 ± 0.3	27.3 ± 0.2
Chicken legs raw material				
-	76.7 ± 0.08	6.3	49.2 ± 0.3	25.1 ± 0.2
Marinated chicken legs				
Control	78.0 ± 0.5	6.5	53.6 ± 0.3	27.3 ± 0.2
30	79.6 ± 0.5	6.4	54.7 ± 0.3	24.6 ± 0.2
50	80.8 ± 0.5	6.3	55.4 ± 0.3	22.8 ± 0.2

The results of the studies of the effect of marinades on the functional and technological indicators of broiler chicken meat (Table 4) indicate that the use of marinades based on milk whey allows to increase the WHC of chicken breasts fillets by about 8% and the chicken legs by 6% relative to the control sample.

Heat treatment loss of marinated meat decreased from 2 to 11% (breast fillets) and from 3 to 5% (leg meat) relative to the control sample.

Table 5 shows the formulations of sauces for poultry meat with the addition of lipids of microalgae.

The technology for making seasoning sauces hown in Fig. 4 is similar It is similar to the technology of cold Italian vegetable oil based sauces.

Table 5. Sauces recipes for poultry meat with the addition of microalgae lipids

Raw material and ingredient names	Mass ratio of the ingredients, kg per kg of sauce	
	'Siberian'	'Baltic'
Crushed pine nuts	0.080	-
Olive oil	0.200	0.100
Sunflower oil additioned with microalgal lipids	0.300	0.130
Parmesan cheese	0.100	-
Crushed basil	0.300	0.250
Crushed garlic	0.020	0.020
Sour cream 15% fat	-	0.500

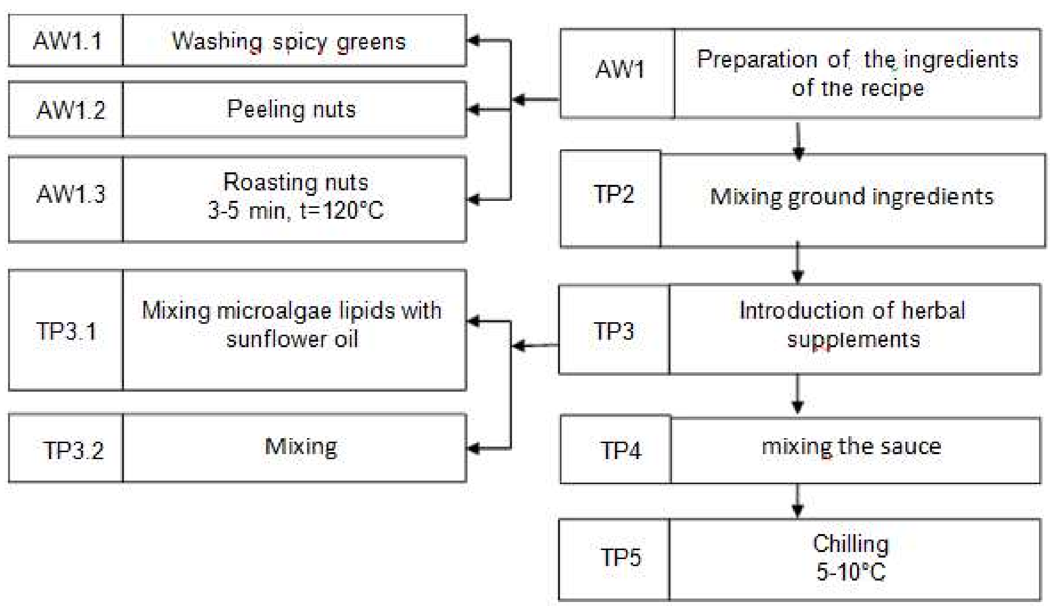

Figure 4. Technological operations for the preparation of seasoning sauces with phytoadditives of *C. sorokiniana* lipid extract. TP – technological process, AW – auxiliary work.

CONCLUSIONS

Aquatic ecosystems are the main birthplace of long-chain ω-3 PUFAs synthesized by microalgae (Gladyshev et al., 2012). Omega-3 fatty acids - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), synthesized by microalgae via trophic chains, are transmitted to aquatic invertebrates, fish and then, to humans. The use of lipid extracts of microalgae rich in omega-3 is important for achieving a balance of PUFAs in food products, which is recommended by the FAO.

Chlorella sorokiniana has a high growth rate (Belkoura, Benider & Dauta, 1997; Politaeva et al., 2017.). Biomass for research was obtained during the cultivation of microalgae in a laboratory bioreactor when illuminated with a fluorescent lamp (2,500 ± 300 Lx), while the specific growth rate during the linear phase of biomass growth was $\mu = 0.26$ day^{-1}. From 1 liter of biomass, 0.60 ± 0.05 g of air-dry biomass was obtained.

The proposed technology for the extraction of lipids from lyophilized biomass of C. sorokiniana microalgae allowed obtaining samples of lipid extracts with lipid yield from 13.5 to 20% of dry biomass, which indicates the high efficiency of lipid extraction regimes (Belkoura et al., 1997; Lizzul et al., 2018).

The fatty acid composition of the isolated lipids of C. sorokiniana microalgae indicates their high nutritional value, however, the ratio of the sum of ω-3 and ω-6 acids in the obtained lipid extract determines the expediency of mixing it with edible oils, which are dominated by linoleic acid, for example, sunflower oil Simopoulos, 2002), which will allow to achieve the desired balance (FAO. 2010. Report of an expert consultation. FAO 'Food and Nutrition'. Geneva, 91. Rome).

To obtain the optimal ratio of ω-3 / ω-6, a mixture of sunflower oil and lipid extract of *C. sorokiniana* microalgae at a ratio of 5–10: 1 by weight was proposed for further use for emulsion sauces and marinades making.

A comparative analysis of the volatile aroma-forming acids and carbohydrates content of milk whey samples obtained by acid coagulation of cow's milk with the use of starter cultures was carried out. The effect of propionic acid on the formation of whey flavor has been established. The use of DVS-starter CHN-19 for to produce a serum with improved organoleptic characteristics and a reduced propionic acid content (0.01 g L^{-1}) was aproved.

Studies on the content of volatile aromatic acids and carbohydrates of curd whey samples allowed to determine the effect of propionic acid content on the formation of whey flavor, which is consistent with the data found in literature (Korenman et al., 2006; Boyeva et al., 2007).

Formulations of marinades and seasonings 'Baltic' and 'Siberian' sauces based on curd whey and a supplement of the extract of the lipid extract of the microalga C. sorokiniana were developed and introduced in Gastroman LLC (St. Petersburg).

ACKNOWLEDGEMENTS. The analysis of the organic acid composition of milk whey by HPLC and the fatty acid composition of the lipid extracts of *Chlorella sorokiniana* microalgae using gas chromatography was carried out in the small innovative enterprise 'Analytics. Materials. Technologies' SPbPU.
We would like to express our gratitude to Igor Vadimovich Kruchin-Bogdanov, Director of the small innovative enterprise 'Analytics. Materials. Technologies' 'SPbPU'.

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