

Yogurt Enriched with *Isochrysis galbana*

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Microalgae are a valuable and innovative emerging source of natural nutrients and bioactive compounds that can be used as functional ingredients in order to increase the nutritional value of foods to improve human health and to prevent disease. The marine microalga *Isochrysis galbana* has great potential for the food industry as a functional ingredient, given its richness in ω 3 long chain-polyunsaturated fatty acids (LC-PUFAs), with high contents of oleic, linoleic, alpha-linolenic acid (ALA), stearidonic, and docosahexaenoic (DHA) acids. This study focuses on the formulation of a functional food by the incorporation of 2% (w/w) of *I. galbana* freeze-dried biomass and 2% (w/w) of *I. galbana* ethyl acetate lipidic extract in solid natural yogurts preparation. In the functional yogurt enriched with microalgal biomass, the ω 3 LC-PUFA's content increased (to 60 mg/100 g w/w), specifically the DHA content (9.6 mg/100 g ww), and the ω 3/ ω 6 ratio (augmented to 0.8). The *in vitro* digestion study showed a poor bioaccessibility of essential ω 3 LC-PUFAs, wherein linoleic acid (18:2 ω 6) presented a bioaccessibility inferior to 10% and no DHA or eicosapentaenoic acid (EPA) was detected in the bioaccessible fraction of the functional yogurts, thus indicating a low accessibility of lipids during digestion. Notwithstanding, when compared to the original yogurt, an added value novel functional yogurt with DHA and a higher ω 3 LC-PUFAs content was obtained. The functional yogurt enriched with *I. galbana* can be considered important from a nutritional point of view and a suitable source of essential FAs in the human diet. However, this needs further confirmation, entailing additional investigation into bioavailability through *in vivo* assays.

Isochrysis galbana

ω 3 long chain-polyunsaturated fatty acids

functional ingredient

yogurt

bioaccessibility

1. Introduction

Nowadays, consumers have become more conscious about food ingredients, which has led to a growing demand for healthy natural products, and reinforced microalgae as an emerging and rich source of nutrients to be used in food supplementation ^[1]. There has been an increasing interest in ω 3 long-chain (LC) polyunsaturated fatty acids (PUFAs) for nutritional and pharmaceutical applications. The nutritional importance of ω 3 LC-PUFAs, mainly eicosapentaenoic acid (EPA, 20:5 ω 3) and docosahexaenoic acid (DHA, 22:6 ω 3), for human health is well established. Nevertheless, since humans cannot synthesize, in adequate levels, fatty acids with more than 18 carbons, they must be obtained from seafood, which is the major source of LC-PUFAs, particularly EPA and DHA ^[2]. Several studies have shown that EPA and DHA play an important role in the functional growth of brain cells, in preventing/reducing cardiovascular and inflammatory diseases, and also in preventing the progression of some types of cancer ^{[1][2][3][4][5]}. DHA is the predominant synaptosomal plasma membrane LC-PUFA in the brain,

important for the normal neurological development. DHA has also been associated with positive effects on memory-related learning ability in Alzheimer's disease [4][6].

LC-PUFAs constitute a large share of marine algal lipids, with planktonic algae being the source of most ω 3 FAs in fish [7]. There has been an increasing interest in microalgal lipids mainly because of their ability to synthesize high quantities of LC-PUFAs, as they are in fact the primary producers of ω 3 LC-PUFAs, since they contain the necessary enzymes [8]. Microalgal lipids are divided into neutral lipids (triacylglycerols, diacylglycerols, and sterol esters), mainly located in lipid droplets in the cytoplasm or plastids, and polar lipids (phospho- and glycolipids), which build the fabric of cellular membranes [8]. The studied marine microalga *Isochrysis galbana* is a highly valuable source of natural bioactive compounds with important biological activities, such as hypocholesterolemic action [9]. The biomass of *I. galbana* is promising as a functional ingredient due to its considerably lipid content (20–30% dw) and richness of ω 3 LC-PUFAs (mainly EPA and DHA) [2][10]. In addition, this marine microalga can provide highly valuable biological compounds, such as sterols, tocopherols, and fucoxanthin [2][9][11].

The change in dietary patterns in the human population, which has been particularly intense in the Western world, has led to an increase in ω 6 FA consumption and a decrease in ω 3 FA consumption, thus leading to an imbalance in the ω 3/ ω 6 ratio level (desirably > 1). Very low ω 3/ ω 6 ratios promote cardiovascular and inflammatory and autoimmune diseases, whereas increased levels of ω 3 LC-PUFAs exert beneficial effects [12]. Given this background, efforts have been made to replace part of the vegetable or animal fat with marine lipids in foods such as mayonnaise, milk, bread, salad dressing, spreads, and yogurts [13]. Most food products have been prepared with fish oil, but, more recently, functional foods with a high content of algal ω 3 LC-PUFAs have been tested, thus eliciting an industrial effort to produce such nutraceuticals. Moreover, the market for microalgae-containing foods has been expanding [14]. For instance, the microalgae *Arthrospira platensis*, *Chlorella vulgaris*, and *I. galbana* have been previously added as functional ingredients to biscuits [11][15], bread [16], and pasta [17].

Yogurt, one of the most consumed fermented dairy products in the world, is able to ensure the daily intake of nutrients and to bring positive impacts on consumers' health due to its active cultures that promote healthy digestion and boost the immune system, providing health benefits [18]. Therefore, yogurt is an ideal vehicle to incorporate ω 3 LC-PUFAs [19]. Dairy products such as yogurts have shown a high potential as carriers of microalgal biomass, ensuring a high share of microalgae ω 3 LC-PUFAs and their bioaccessibility, given yogurt's chemical and rheological properties, easiness to incorporate emulsions, and oxidative stability [3][20]. In fact, various yogurt products containing DHA have already been developed and marketed [19].

The aim of this work was to formulate a high-value functional food by the incorporation of freeze-dried biomass and ethyl acetate lipidic extract of *Isochrysis galbana* in commercial plain yogurt in order to increase ω 3 LC-PUFAs content (mainly DHA) and to enhance ω 3 LC-PUFAs bioaccessibility. Thus, based on the study's results, the formulation of the functional yogurt could be optimized for maximal bioavailability of ω 3 LC-PUFAs.

2. Yogurt Enriched with *Isochrysis galbana*

The microalga incorporation level of 2% (w/w) in the solid yogurts was chosen based on the literature available over microalgal biomass incorporation into food products [11][15][17], in order to not compromise the sensory acceptability of the final product in terms of color, fishy flavor and odor. The functional yogurts with 2% (w/w) of *I. galbana* of freeze-dried biomass and 2% (w/w) of *I. galbana* ethyl acetate extract incorporation presented an innovative green tonality (Figure 1).

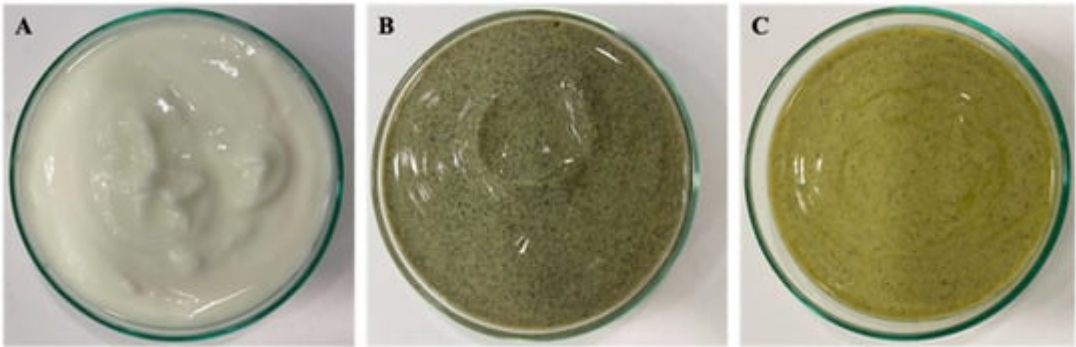


Figure 1. Yogurt products preparation: (A) Control Yogurt; (B) Yogurt with 2% (w/w) of *I. galbana* freeze-dried biomass; (C) Yogurt with 2% (w/w) of *I. galbana* ethyl acetate extract.

The sensory attributes of the novel functional yogurt and the consumer’s acceptance need further evaluation.

2.1. Proximate Composition

The lipid bioaccessibility of the functional yogurts was high (exceeding 86%). In the control yogurt and both functional yogurts, the palmitic acid (16:0) was highly bioaccessible (>100%). Oleic acid (18:1 n-7) was more bioaccessible in the yogurt with ethyl acetate extract (18 ± 0.9%). Among the chain FA, palmitic acid (16:0) followed the pattern of bioaccessibility enhancement after *I. galbana* freeze-dried biomass and ethyl acetate extract incorporation was mainly yogurt matrix, which was also verified by Paul et al. [26] study, which used the microalga *Aureocoryanum* sp. in the development of a skimmed functional yogurt. Linoleic acid (18:2 n-6) showed a low content was 21.4 ± 0.9% dw. The observed proximate composition in the studied microalgal biomass is similar to that reported by other authors [10][17][21][22][23]. No DHA or EPA content was found to be bioaccessible in the functional yogurts, despite their initial presence.

Table 1. Proximate composition (%) of *I. galbana* freeze-dried biomass, *I. galbana* ethyl acetate extract, control yogurt, yogurt with 2% (w/w) of *I. galbana* freeze-dried biomass, and yogurt with 2% (w/w) of *I. galbana* ethyl acetate extract. The observed bioaccessibility loss as a result of *I. galbana* incorporation is unexpected, since various studies considered yogurts an ideal food matrix for incorporating ω3 LC-PUFAs, making them more bioaccessible/bioavailable due to the preformed emulsions [3][27][28]. In view of the low bioaccessibility of ω3 LC-

Proximate Composition	<i>I. galbana</i> Freeze-Dried Biomass	<i>I. galbana</i> Ethyl Acetate Extract	Control Yogurt	Yogurt with <i>I. galbana</i> Freeze-Dried Biomass	Yogurt with <i>I. galbana</i> Ethyl Acetate Extract
	(% Dry Weight)	(% Dry Weight)	(% Wet Weight)	(% Wet Weight)	(% Wet Weight)
Moisture	7.6 ± 0.1	-	87.9 ± 0.1 ^a	86.7 ± 0.0 ^b	87.8 ± 0.1 ^a
Ash	14.6 ± 0.0	-	0.7 ± 0.0 ^a	1.0 ± 0.0 ^b	0.7 ± 0.0 ^a

Proximate Composition	<i>I. galbana</i> Freeze-Dried Biomass	<i>I. galbana</i> Ethyl Acetate Extract	Control Yogurt	Yogurt with <i>I. galbana</i> Freeze-Dried Biomass	Yogurt with <i>I. galbana</i> Ethyl Acetate Extract
	(% Dry Weight)	(% Dry Weight)	(% Wet Weight)	(% Wet Weight)	(% Wet Weight)
Protein	38.7 ± 0.0	-	3.2 ± 0.1 ^a	4.0 ± 0.1 ^b	3.2 ± 0.1 ^a
Lipid	24.5 ± 0.6	21.4 ± 0.9	2.3 ± 0.3 ^a	2.7 ± 0.0 ^a	2.6 ± 0.1 ^a

ants, thus ents [29]. and other for non- proteins and [8][10]. This was verified by Zhang *et al.* [30], who reported that some of the FA in the microalga *Chlorella* were attached to the cell wall and linked to carbohydrates by an ether bond. Therefore, since microalgal polar lipids are located in the significant differences ($p < 0.05$) between proximate composition of yogurt samples. cell membrane and in *I. galbana*, the main portion of DHA was found to be present in the polar fraction; this can

2.2 Lipid Classes

explain why the ω 3 LC-PUFA was not bioaccessible.

The lipid class distribution before and after digestion (bioaccessible fraction) of *I. galbana* freeze-dried biomass, *I. galbana* ethyl acetate extract, control yogurt and functional yogurts is presented in Table 2. Since DHA and other ω 3 LC-PUFAs are highly unsaturated, this may lead to a lower bioaccessibility percentage [29], which can also explain the low fatty acid bioaccessibility detected in this study.

Table 2. Lipid class distribution (% of total lipid) before and after digestion (bioaccessible fraction) of *I. galbana* freeze-dried biomass, *I. galbana* ethyl acetate extract, control yogurt, yogurt with 2% (w/w) of *I. galbana* freeze-dried biomass and yogurt with 2% (w/w) of *I. galbana* ethyl acetate extract. A low lipid bioaccessibility means a lower nutritional value regarding ω 3 LC-PUFAs content in the functional yogurts, but to achieve a higher level of bioaccessible LC-PUFAs, a much higher quantity of *I. galbana* freeze-dried biomass and ethyl acetate extract added to the yogurt than only 2% w/w would be needed. This would be

unfeasible because of the impact on sensory properties. Therefore, it is important to find solutions to enhance the

Sample		Lipid Classes			
		TAG ¹	FFA ²	Polar Lipids	Sterol
<i>I. galbana</i> freeze-dried biomass	Initial	36.8 ± 3.1 ^{aA}	32.6 ± 1.6 ^{aA}	14.8 ± 2.3 ^{aA}	15.9 ± 0.9 ^{aAB}
	Bioaccessible	nd ^{bλ}	53.9 ± 4.8 ^{bλ}	22.2 ± 2.6 ^{bλ}	23.9 ± 5.1 ^{aλ}
<i>I. galbana</i> ethyl acetate extract	Initial	18.1 ± 1.1 ^B	55.8 ± 0.1 ^B	13.7 ± 0.0 ^A	12.5 ± 1.2 ^A
	Bioaccessible	-	-	-	-
Control Yogurt	Initial	47.1 ± 0.2 ^{aC}	25.2 ± 0.3 ^{aC}	12.7 ± 0.0 ^{aAB}	15.0 ± 0.2 ^{aA}
	Bioaccessible	nd ^{bλ}	46.3 ± 1.8 ^{bλ}	28.8 ± 1.6 ^{bλ}	24.9 ± 0.2 ^{bλ}
Yogurt with <i>I. galbana</i> freeze-dried biomass	Initial	59.1 ± 0.3 ^{aC}	12.9 ± 1.1 ^{aD}	20.2 ± 2.0 ^{aAC}	7.8 ± 2.1 ^{aAC}
	Bioaccessible	nd ^{bλ}	44.7 ± 4.5 ^{bλ}	32.1 ± 3.2 ^{bφλ}	23.2 ± 3.9 ^{bλ}
Yogurt with <i>I. galbana</i> ethyl acetate extract	Initial	52.8 ± 2.2 ^{aAC}	16.5 ± 2.3 ^{aD}	18.0 ± 2.2 ^{aAD}	12.8 ± 2.2 ^{aA}
	Bioaccessible	nd ^{bλ}	35.3 ± 2.0 ^{bφ}	39.6 ± 5.8 ^{bφ}	25.1 ± 4.9 ^{bλ}

biomass in yogurts was shown to be more effective in enhancing ω 3 LC-PUFAs content (mainly DHA) than the ethyl acetate extract incorporation, which means that the green solvent lipid extraction from *I. galbana* was not as

2.3. Fatty Acid Profile

The FA composition (in % of total FA and in mg/100 g DW) of the studied *F. garibana* freeze-dried biomass and ethyl acetate lipidic extract is shown in Table 3.

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Fatty Acid	<i>I. galbana</i> Freeze-Dried Biomass		<i>I. galbana</i> Ethyl Acetate Extract		Control Yogurt		Yogurt with <i>I. galbana</i> Freeze-Dried Biomass		Yogurt with <i>I. galbana</i> Ethyl Acetate Extract	
	% Total Fatty Acids	mg/100 g Dry Weight	% Total Fatty Acids	mg/100 g Dry Weight	% Total Fatty Acids	mg/100 g Wet Weight	% Total Fatty Acids	mg/100 g Wet Weight	% Total Fatty Acids	mg/100 g Wet Weight
20:1 ω11	0.9 ± 0.1 ^a	175 ± 9 ^A	0.8 ± 0.0 ^a	136 ± 1 ^B	0.2 ± 0.1 ^b	4.0 ± 1.7 ^C	0.3 ± 0.0 ^b	7.1 ± 0.5 ^C	0.2 ± 0.1 ^b	4.5 ± 1.9 ^C
22:1 ω11	0.5 ± 0.0 ^a	97 ± 3 ^A	0.1 ± 0.0 ^b	15 ± 0 ^B	nd ^b	nd ^B	nd ^b	nd ^B	nd ^b	nd ^B
Σ MUFA ₂	28.7 ± 0.0 ^a	5497 ± 8 ^A	27.9 ± 0.4 ^a	4932 ± 71 ^B	24.0 ± 0.6 ^b	401 ± 8 ^C	25.7 ± 0.9 ^b	560 ± 19 ^D	24.7 ± 0.7 ^b	502 ± 15 ^D
16:2 ω4	0.3 ± 0.0 ^a	57 ± 1 ^A	0.4 ± 0.0 ^b	65 ± 0 ^B	0.2 ± 0.0 ^c	2.9 ± 0.1 ^C	0.2 ± 0.0 ^c	3.4 ± 0.1 ^C	0.2 ± 0.0 ^c	3.4 ± 0.8 ^C
18:2 ω6	12.1 ± 0.1 ^a	2313 ± 14 ^A	10.2 ± 0.2 ^b	1806 ± 31 ^B	2.2 ± 0.1 ^c	40 ± 1 ^C	3.0 ± 0.1 ^d	65 ± 2 ^C	2.6 ± 0.1 ^e	54 ± 2 ^C
18:3 ω3	11.8 ± 0.1 ^a	2260 ± 10 ^A	10.1 ± 0.1 ^b	1779 ± 17 ^B	0.6 ± 0.0 ^c	11.5 ± 0.7 ^C	1.4 ± 0.1 ^c	30 ± 2 ^C	0.8 ± 0.6 ^c	16.9 ± 12.2 ^C
20:4 ω3	0.2 ± 0.0 ^a	35 ± 0 ^A	0.2 ± 0.0 ^{ab}	30 ± 0 ^{AB}	nd ^b	nd ^B	nd ^b	nd ^B	nd ^b	nd ^B
20:4 ω6	0.3 ± 0.0 ^a	66 ± 2 ^A	0.2 ± 0.0 ^b	39 ± 1 ^B	0.1 ± 0.0 ^c	2.0 ± 0.2 ^C	0.1 ± 0.0 ^c	2.8 ± 0.3 ^C	0.1 ± 0.0 ^c	2.7 ± 0.2 ^C
18:4 ω3	10.2 ± 0.0 ^a	1957 ± 3 ^A	7.9 ± 0.2 ^b	1387 ± 29 ^B	0.6 ± 0.0 ^c	10.1 ± 0.5 ^C	0.8 ± 0.1 ^d	16.7 ± 1.2 ^C	0.5 ± 0.0 ^c	9.4 ± 0.8 ^C
20:5 ω3	1.2 ± 0.0 ^a	234 ± 1 ^A	1.0 ± 0.2 ^a	182 ± 28 ^B	nd ^b	nd ^C	0.1 ± 0.0 ^b	2.8 ± 0.3 ^C	0.1 ± 0.0 ^b	2.5 ± 0.0 ^C
22:5 ω3	0.2 ± 0.0 ^a	35 ± 1 ^A	0.1 ± 0.0 ^b	22 ± 0 ^B	nd ^b	nd ^B	nd ^b	nd ^B	nd ^b	nd ^B
22:5 ω6	1.9 ± 0.0 ^a	358 ± 5 ^A	1.2 ± 0.0 ^b	220 ± 7 ^B	nd ^c	nd ^C	nd ^c	nd ^C	nd ^c	nd ^C
22:6 ω3	8.6 ± 0.2 ^a	1637 ± 30 ^A	5.8 ± 0.1 ^b	1021 ± 18 ^B	nd ^c	nd ^C	0.4 ± 0.1 ^d	9.6 ± 1.0 ^C	0.3 ± 0.0 ^{cd}	6.2 ± 0.6 ^C
Σ PUFA ₃	47.9 ± 0.1 ^a	9154 ± 14 ^A	37.8 ± 0.5 ^b	6686 ± 90 ^B	4.4 ± 0.1 ^c	89 ± 2 ^C	6.7 ± 0.2 ^d	146 ± 7 ^C	5.2 ± 0.4 ^c	107 ± 8 ^C

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Fatty Acid	<i>I. galbana</i> Freeze-Dried Biomass		<i>I. galbana</i> Ethyl Acetate Extract		Control Yogurt		Yogurt with <i>I. galbana</i> Freeze-Dried Biomass		Yogurt with <i>I. galbana</i> Ethyl Acetate Extract	
	% Total Fatty Acids	mg/100 g Dry Weight	% Total Fatty Acids	mg/100 g Dry Weight	% Total Fatty Acids	mg/100 g Wet Weight	% Total Fatty Acids	mg/100 g Wet Weight	% Total Fatty Acids	mg/100 g Wet Weight
Σω3	32.5 ± 0.1 ^a	6217 ± 16 ^A	25.1 ± 0.1 ^b	4426 ± 15 ^B	1.2 ± 0.0 ^c	22 ± 1 ^C	2.7 ± 0.2 ^d	60 ± 4 ^D	1.6 ± 0.4 ^c	32 ± 9 ^C
Σω6	14.9 ± 0.0 ^a	2847 ± 1 ^A	12.2 ± 0.6 ^b	2159 ± 104 ^B	2.7 ± 0.1 ^c	48 ± 2 ^C	3.4 ± 0.1 ^d	74 ± 1 ^C	3.1 ± 0.1 ^c	63 ± 2 ^C
Σω3/Σω6	2.2 ± 0.0 ^a	2.2 ± 0.0 ^A	2.1 ± 0.1 ^a	2.1 ± 0.1 ^A	0.4 ± 0.0 ^b	0.4 ± 0.0 ^B	0.8 ± 0.1 ^c	0.8 ± 0.1 ^C	0.5 ± 0.1 ^b	0.5 ± 0.1 ^B

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2.3.12. Fatty Acid Profile of the Functional Yogurts

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