



Effect of Spirulina Biomass Fortification for Biscuits and Chocolates

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 03/09/2018 Accepted : 02/04/2019</p> <p>Keywords: <i>Arthrospira platensis</i> <i>Spirulina</i> protein Amino acid Biscuit Chocolate</p>	<p>Microalgae can be regarded as an alternative and promising ingredient for food fortification or enrichment. Due to their nutritional composition, especially protein-high composition, they considered as a sustainable protein source for food. The aim of this work was to evaluate the <i>Arthrospira platensis</i> (<i>Spirulina</i>) dry biomass which is known as the greater protein and amino acid source in blue-green microalga, as innovative ingredient for newly formulated and produced biscuits and chocolates, for baby and children diets. All samples with <i>Spirulina</i> biomass showed significantly higher protein and amino acid content.</p>

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Introduction

Microalgae have been used in human and animal nutrition for many years and received an increasing interest nowadays due to their nutraceutical and therapeutic functions (Spolaore et al., 2006; Vigani et al., 2015; Lucas et al., 2018). *Spirulina*, known as *Arthrospira platensis* and *Arthrospira maxima*, is the most commonly and commercially used blue-green microalgae also classified as cyanobacteria (Nicholetti, 2016; Garcia et al., 2017). These blue-green microalgae have been attracting the most attention due to be a promising source for protein and other biologically active functional ingredients, especially high biological valued protein (60-70 g/100g), with significant bioactive compounds such as, chlorophylls, carotenoids, phycobiliproteins, polyunsaturated fatty acids, extracellular polysaccharides etc. (Belay et al., 1993; Belay, 2002; Bhowmik et al., 2009; Hoseini et al., 2013a; Hoseini et al., 2013b). Chlorophyll has been shown to stimulate liver function recovery and increase bile secretion (Bishop and Zubeck, 2012). Phycobiliproteins are hydrophilic proteins bonded to the photosynthetic pigments phycobilins, mainly found in cyanobacteria and is exploited for many purposes (e.g. natural dyes,

fluorescent agents/markers, cosmetics) but as nutraceutical it has been commercialized as antioxidant, anti-inflammatory, neuroprotective or hepatoprotective agent (Sonani et al., 2016; Garcia et al., 2017). *Spirulina* is a producer of structurally diverse exopolysaccharides (EPS) that have multiple pharmaceutical activities, such as, antioxidant, antitumor, antihyperlipidemic, antibacterial and anticoagulant activities (Liu et al., 2016). According to these biological functions, *Spirulina* is noticed as highlighting antioxidant, anti-inflammatory, antimicrobial activities and slow the progression of certain cancers and reduce the risks of cardiovascular disease, neurodegenerative diseases and diabetes (Plaza et al., 2010; Garcia et al., 2017). Also, *Spirulina* has been approved as “safe food” with no toxicological effects and has received GRAS (Generally Recognized as Safe) certification (Lucas et al. 2017), with a daily consumption of 5-10 grams and notified that can cure a malnourished child by taking a daily dose of 1-3 grams. These advantages make *Spirulina* a good raw material and ingredient for food production.

Biscuits and chocolates are the most consumed snack food in every age, although their low protein content and high unhealthy ingredient presence. In recent years, fortification and/or enrichment of nutritional value for foods especially for children diet, is gained an increasing interest (Potter et al., 2013; Rathod and Annapure, 2016). Childhood snacking trends are moving toward three snacks per day with over 27% of daily calories coming from snacks. The largest increments have been in salty snack and candy consumption, while desserts and sweetened beverages remain the major sources of calories from snacks. The nutrient density index of added sugars and saturated fat is very high in Australia, Mexico, and the US. Also, in Turkey, 31% have fast food at least once every day and 61% skip meals and more than one third chose fast food or cakes and sweets as snacks between meals (Akman et al., 2010; Piernas and Popkin, 2010; Chambers et al., 2016). Among these previous studies, due to snack food consumptions of children and adults, enrichment of snack foods become an emerging trend.

The presented study is aimed to evaluate the effects of a 2% (w/w) *Spirulina* biomass addition on nutritional value of chocolate and biscuits formulated for babies and children. Despite many studies have been focused on nutritional and textural properties on microalgae-added food products (Gouveia et al., 2008; Figueira et al., 2011; Dinu et al., 2012; Achour et al., 2014; Abd El Baky et al., 2015; Singh et al., 2015; Ak et al., 2016; Batista et al., 2017; Garcia-Segovia et al., 2017), this paper is the first one focusing on the amino acid profile. Also, it can be stated that, this work represents the first example in the literature to follow protein and amino acids content together of the home-made biscuits and chocolates for baby and children diets.

Materials and Methods

Materials

Spirulina biomass was obtained from the cultivation of *Arthrospira platensis* in a laboratory scale, with a 2L photobioreactor at 25°C for 25-day cultivation, harvested by filtration and freeze dried. Chemical composition of the dry biomass used is as; protein 65±0.09 g.100g⁻¹, fat 2.5±1.45 g.100g⁻¹, total phenolic 1.39±0.14 mg-GAE.g⁻¹, antioxidant activity 1.10±0.07 µg-TE.g⁻¹. The dried-biomass was stored in dry and cool conditions at 4°C. All reagents to perform the analysis were purchased from Merck (Germany).

Methods for Production of Biscuits and Chocolate

The formulas used in the presenting study were collected from 0-6 ages children mothers and were not commercially produced. Chocolate and biscuit recipes were used in conclusion after many home-made recipes using for non-gluten and non-sugar added diets. After several sensory analysis which were performed with mothers who have children with the age of 2-12, chocolate and biscuit recipes were chosen as described below;

Chocolate samples were prepared using 30% coconut butter, 25% cacao, %45 grape molasses and biscuits were prepared using 28% whole wheat flour, 10 % semolina, 25% oat flour, 13% butter, 14% yoghurt and 10% yoghurt juice(water). 2 g *Spirulina* biomass was added to all samples. The chocolate samples were left to rest at

refrigerator for 2 h after mixed and given shape. Biscuit samples were baked in an oven at 175°C during 25 min. samples were stored at -80°C until analysed.

2 g *Spirulina* biomass was added to all samples per 100 g, which is selected best after sensory analysis of pre-studies (data not shown). Also, each serve portions of samples are 10 g.

Methods for Analysis

Protein was determined by the Kjeldahl method according to AOAC (2005) and multiplied by a conversion factor of 5,7 to obtain the protein content. Fat content of products was determined by Roese-Gottlieb method (AOAC, 2005) by extraction using petroleum ether and diethyl ether.

Amino acid analysis was carried out by ion-exchange chromatography. The amino acids for chocolate samples were obtained by peptide hydrolysis with 6 mol.L⁻¹ HCl at 110±1°C for 22 h and purified with Amberlite IR-120. Tryptophan was determined with the same method, but the hydrolysis was carried separately using 4 mol.L⁻¹ LiOH at 110±1°C for 24 h (Penke et al., 1974). Each amino acid was detected based on the retention time of external standards for the individual amino acids. Amino acids concentrations were calculated based on the area under the peak established for a given amino acid (Cohen et al., 1989). The results were expressed as g/100g of the sample.

The amino acid profile of the biscuits and biomass was determined by Ion Exchange Chromatography (IEC) according to the method of Hussain (1998), using the Technicon Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The samples were defatted, hydrolysed, evaporated in a rotary evaporator before being injected into the Amino Acid Analyzer for the determination of their amino acid profile. The period of analysis for each sample was 72 min and the net height of each peak produced by the chart recorder of the (TSM) (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of triplicate determinations. Norleucine was used as the internal standard.

Statistical Analysis

The experiment was carried out in 3 replicates. Statistical significance among samples was performed using one-way-ANOVA (Da Silva et al. 2007). Differences were considered significant at p<0.05 level.

Results and Discussions

Microalgae have been commercially utilized frequently, and the mainly-used genera are *Dunaliella*, *Chlorella*, and *Arthrospira* (*Spirulina*) for functional foods. Besides these genera, *Isochrysis*, *Phaeodactylum* and *Haematococcus* are also added to food products for the fortification of nutritional and sensorial properties (Beheshtipour et al., 2012). Food fortification and enrichment using microalgae have shown improvements in different nutritional and structural parameters for the last ten years (Figueira et al., 2011, DeMarco et al., 2014).

In the presenting paper, the results pertaining to analysis of *Spirulina* biomass, control samples and biomass added chocolate and biscuits samples were given in Table 1 and Table 2, respectively.

Table 1 Protein and fat content (g/100g) and amino acid composition (g/100g) of *Spirulina* and Chocolate samples

Component	Spirulina	Chocolate	Chocolate with 2% Spirulina
Protein	65±0.09	5.09±0.01	11.81±0.01 ^a
Fat	2.5±0.45	41.23±0.05	36.25±0.02 ^a
Alanine	4.28±0.10	0.20±0.02	0.38±0.02 ^a
**Arginine	4.15±0.04	0.37±0.03	0.87±0.03 ^a
Aspartic Acid	5.79±0.03	0.23±0.08	0.79±0.02 ^a
Glutamic Acid	8.39±0.02	0.52±0.05	0.76±0.05 ^a
Glycine	3.09±0.05	0.59±0.04	0.76±0.09 ^a
**Histidine	1.18±0.02	0.05±0.18	0.11±0.02 ^a
*Isoleucine	3.02±0.02	0.25±0.02	0.74±0.02 ^a
*Leucine	4.49±0.01	0.22±0.03	0.64±0.03 ^a
*Lysine	2.98±0.08	0.02±0.03	0.17±0.01 ^a
*Methionine	1.06±0.06	0.02±0.02	0.13±0.01 ^a
*Phenylalanine	2.37±0.05	0.09±0.02	0.17±0.01 ^a
*Threonine	2.98±0.06	0.20±0.03	0.44±0.02 ^a
Serine	2.98±0.06	0.16±0.03	1.47±0.06 ^a
Tyrosine	2.48±0.04	0.16±0.01	0.27±0.02 ^a
*Valine	3.15±0.01	0.03±0.02	0.47±0.02 ^a

Means ± standard deviation (n=2). Control vs 2% Spirulina-fortified sample: ^a(P=0.002). *essential amino acids, **important amino acids for infant and children

Table 2 Protein and fat content (g/100g) and amino acid composition (g/100g) of *Spirulina* and Biscuit samples

Component	Spirulina	Biscuit	Biscuit with 2% Spirulina
Protein	65±0.09	9.67±0.03	18.29±0.04 ^a
Fat	2.5±0.45	32.70±0.03	26.54±0.02 ^a
Alanine	4.28±0.10	0.64±0.02	1.91±0.02 ^a
**Arginine	4.15±0.04	0.36±0.01	0.81±0.04 ^a
Aspartic Acid	5.79±0.03	0.71±0.01	1.37±0.02 ^a
Glutamic Acid	8.39±0.02	0.52±0.01	0.75±0.02 ^a
Glycine	3.09±0.05	0.63±0.01	1.07±0.05 ^a
**Histidine	1.18±0.02	0.07±0.02	0.43±0.01 ^a
*Isoleucine	3.02±0.02	0.21±0.02	1.20±0.01 ^a
*Leucine	4.49±0.01	0.26±0.02	1.28±0.02 ^a
*Lysine	2.98±0.08	0.28±0.03	0.49±0.01 ^a
*Methionine	1.06±0.06	0.09±0.01	0.25±0.02 ^a
*Phenylalanine	2.37±0.05	0.05±0.01	0.26±0.02 ^a
*Threonine	2.98±0.06	0.63±0.01	1.07±0.04 ^a
Serine	2.98±0.06	0.81±0.02	1.63±0.03 ^a
Tyrosine	2.48±0.04	0.26±0.03	0.36±0.01 ^a
*Valine	3.15±0.01	0.59±0.03	0.86±0.03 ^a

Means ± standard deviation (n=2). Control vs 2% Spirulina-fortified sample: ^a(P=0.008), *essential amino acids, **important amino acids for infant and children

Because of good acceptance of taste, versatility, convenient consumption due to their ease of conservation and transportation, texture, and appearance, cookies and biscuits are easy-to-adapt microalgae-based ingredients. First studies have begun three-decades ago with *Dunaliella* species added to breads (Finney et al., 1984). Finney noticed that, *Dunaliella* microalgae can be used for protein supplement, although data of bread's protein content did not include in this research.

It is known that, *Spirulina* biomass has a significant amount of protein nearly about 70% of the biomass. Therefore, in this work, in corporation of 2% (w/w) *Spirulina* for was directly positively influenced on the amount of protein and lipid for home-made chocolate and biscuits samples. Despite approximately 12.5% decrease in lipid content for all samples (Table 1 and 2), because of the high percentage of the usage of coconut oil and butter as sources, lipid contents were extremely high for the purpose of healthy-snacks. Also, protein values have shown a significant difference in chocolate (Table 1) and biscuit

(Table 2) samples. From the tables, the protein values for home-made chocolate and biscuit were between the range 11.81 and 18.29 g.100g⁻¹. All chocolate and biscuits which are commercially produced have smaller amount of protein when compared with the labels, as the commercial baby biscuits' protein content is 4.6 g.100 g⁻¹ and commercial chocolate's protein content is about 4.9 g.100 g⁻¹. It can be stated that, home-made chocolate and biscuit with 2% (w/w) *Spirulina* have nearly four-fold protein content comparing with commercial baby foods. This observation is similar to the researches of addition of dry *Spirulina* to yogurt reported by Akalin et al. (2009) and to ice-cream reported by Agustini et al. (2016). Studies using microalgae dry biomass for food enrichment have shown an increase of protein content in cookies (De Marco et al., 2014; Batista et al., 2017) and in gluten-free bread (Figueira et al., 2011; Prabhasankar et al., 2009) noticed that the macroalgae addition to noodles increased a 20% of protein content also support this study findings.

Quality of protein depends on its essential amino acid profile and content. This research work also analysed the amino acid contents of home-made chocolate and biscuit samples. The amino acid results belong to samples, were satisfying the patterns provisioned by FAO/WHO (2005) and after 2% (w/w) *Spirulina* fortification, at least a 15-18% total amino acid content increment were detected. The results for essential amino acid in *Spirulina*, be shown in both tables, exhibited; leucine ($4.49 \pm 0.01 \text{ g.}100\text{g}^{-1}$) and valine ($3.15 \pm 0.01 \text{ g.}100\text{g}^{-1}$) in large proportions, whilst the lowest value was determined for methionine ($1.06 \pm 0.06 \text{ g.}100\text{g}^{-1}$). *Spirulina* has relatively low levels of threonine and lysine compare with amino acid levels of whey, two-fold of increase in both chocolate and biscuit samples were noted with the addition of the biomass. Apart from the 8 essential amino acids (Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine), are very important for adults also Histidine and Arginine are the most essential and important amino acids for infants and children. Histidine content of control samples for both chocolate and biscuits were 0.37 and 0.36 $\text{g.}100\text{g}^{-1}$, and by the 2% (w/w) *Spirulina* addition, this content extremely increased three-fold (0.89 and 0.81 $\text{g.}100\text{g}^{-1}$, respectively) (Table 1 and Table 2). These results confirmed the importance of microalgae have a considerable improvement of amino acid content for home-made chocolate and biscuit.

Whereas *Spirulina* biomass does not contain Tryptophan, this essential amino acid is a required for children aged-2, which is stated from FAO/WHO daily intake content 4 mg.kg^{-1} . Despite the Tryptophan deficiency, Phenylalanine and Tyrosine (aromatic) and Methionine (sulphur) amino acids for enriched chocolates and biscuits were almost fulfil the required daily intake (RDI) as 25 and 15 mg.kg^{-1} , respectively.

It can be safely stated that, *Spirulina* is an important source for natural biocompounds for nutrition. Addition of *Spirulina* to foods can be formulated and designed for infants and children's diet. The home-made biscuits and chocolates designed with 2% (w/w) of *Spirulina*, as a natural ingredient, exhibited adequate protein content and rich amino acid profile with a significant increase in the daily intake of essential amino acids, histidine and arginine, for infants and children. However, these baby snack food formulas fat contents need to be reduced, especially more formulas may be created with low-fat raw ingredients, also detail structural and sensory analysis with the shelf-life determination are needed for future studies.

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