

Microalgal biotechnology for development of energy gel in sports

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<u>Abstract</u>

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Introduction

Biomass of *Spirulina* is a source of protein, minerals, carotenoids, and essential polyunsaturated fatty acids. Due to its nutritional profile, the addition of *Spirulina* in foods as energy gels contributes to the development of innovative products for athletes. Thus, the objective of this study was develop the sensory profile and evaluate the acceptance of energy gels developed with the addition of *Spirulina*. Sensory profile for energy replenisher gel added of *Spirulina* was prepared. The terms relevant to description of the product quality were "artificial acai berry" and "artificial banana" for aroma attribute; "artificial acai berry", "artificial banana" and "*Spirulina*" for flavor attribute; "viscosity" and "adhesiveness" to the texture attribute. The products with *Spirulina* were accepted (74%) by the target public. In addition, 50.5% of panelists had the opinion between "probably and certainly would buy" for the product added of 0.50% (w/w) of *Spirulina*. Thereby, the microalgal biomass can be added to foods for athletes and physical activities practitioners, without affecting the sensory characteristics of this product.

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To keep the energy levels required for training, athletes and physical activities practitioners make use of energy supplements (Sousa *et al.*, 2013). Among these supplements the energy replenishers can be highlighted. These are dietary supplements carbohydrate basis that guarantee caloric intake for physical exercises by maintaining glucose levels. Carbohydrate supplements based liquid or solid state show no difference in glycogen recovery (Pereira and Souza, 2004). However, the energy supplement in gel form avoids gastric discomfort and it is a practical alternative to consumption and transport (Alves *et al.*, 2009).

The genus *Spirulina* is a photoautotrophic filamentous cyanobacterium belonging to the family Oscillatoriaceae, phylum Cyanophyta (Pelizer *et al.*, 2003). *Spirulina* is a microalga with filament has cylindrical, multi-cellular trichomes in an open left-hand helicoidal shape. The typical morphology of *Spirulina* is characterized by its regularly coiled trichome, and the morphological features, such as the diameter of helix, the width of filament, and the degree of spiralization, are the main taxonomic criteria for *Spirulina* (Lewin, 1980; Ciferri, 1983).

Biomass of Spirulina has nutritional profile

that makes it ideal as a food supplement, making their addition interesting in energy replenishers. Consumption of *Spirulina* is legally authorized in several countries by Food and Drug Administration (FDA, 2012). Thus, *Spirulina* has been studied for use in human food (Carvalho *et al.*, 2016; Santos *et al.*, 2016).

Spirulina may have up to 86% (w/w) protein content (Morais *et al.*, 2009), with digestibility to 74% (w/w) (Lisboa *et al.*, 2016) and around 20% (w/w) of carbohydrates (Moreira *et al.*, 2016). Spirulina is also a source of minerals, carotenoids, pigments and essential polyunsaturated fatty acids, such as γ -linolenic (Peiretti and Meineri, 2011; Kumar *et al.*, 2013). In this context, it is important to study the development of new energy gels with Spirulina because this product has a differential to improve the performance of athletes.

There are many marks of this supplement (energy gel) available on the market, however, none of these products was developed with the addition of *Spirulina*. Moreover, it was not proposed a profile of sensory attributes for this food, and was not evaluated the acceptance of this type of product by potential consumers.

In modern industrial companies, all activities are developed around preference of consumers.

In this background, sensory evaluation has been widely used in many sectors such as food (Zeng *et al.*, 2008). Sensory analysis presents techniques that find applications in various stages of preparation of food products, contributing to the quality and acceptance of these products. Subjective methods evaluate consumer opinion through your choice and/ or acceptance of a product (Queiroz and Treptow, 2006).

The descriptive sensory techniques are used to profile a product on all its perceived sensory characteristics. Quantitative descriptive analysis (QDA) is one of the most common descriptive sensory techniques used to describe the nature and the intensity of sensory properties from a single evaluation of a product. QDA measures the intensity with which the sensory characteristics of a product were perceived by panelists (Stone *et al.*, 1974; Ng *et al.*, 2012).

The objective of this study was to develop the sensory profile and evaluate the acceptance of energy gels developed with the addition of *Spirulina*.

Materials and Methods

Biomass of Spirulina

The microalga added to foods was *Spirulina* sp. LEB 18 (Morais *et al.*, 2008), which was produced at a pilot plant for *Spirulina* production. The pilot plant is located on the shores of Mangueira Lagoon (33° 30' 13'' S and 53° 08' 59'' W) in Santa Vitória do Palmar, RS, Brazil. The unit consists of three open tanks of 10000 L raceway type and one open tank of 1000 L raceway type for inoculums propagation. The cultivations were protected by tunnel transparent film with UV protection and exposed to natural environmental conditions (Morais *et al.*, 2009).

When the concentration reached *Spirulina* 0.50 g/L, the biomass was harvested by filtration, extruded and drought in tray driers. *Spirulina* biomass after drying was kept under freezing and then, ground in a ball mill (Model Q298, Quimis, Brazil) and sieved with sieve shaker to achieve 0.053 mm of diameter. This biomass in the form of powder was packaged to vacuum and stored for further addition in the formulations.

Development of energy gel samples

The developed samples contained the following ingredients: maltodextrin, water, fructose, sodium chloride, magnesium chloride, sodium citrate, citric acid, fumaric acid, pectin, flavors (banana and acai berry), potassium citrate, sodium benzoate, potassium sorbate, colorants and *Spirulina* biomass.

The selection of ingredients employed was based on literature (ANVISA, 2010) and similar products available on the market.

In this study, three formulations were developed: A (without addition of *Spirulina* and 0.25% w/w pectin); A₁ (0.35% w/w *Spirulina* and 0.25% w/w pectin) and A₂ (0.50% w/w *Spirulina* and 0.50% w/w pectin). The sample B (commercial sample without *Spirulina* in its composition) was acquired in the local market of the city of Rio Grande-RS, Brazil.

The selected ingredients were weighted, homogenized and brought to boiling. The infrastructure used for the development of products was the Center for Development of Food with *Spirulina* (CEAS), installed on the Laboratory of Biochemical Engineering (LEB) of the Federal University of Rio Grande (FURG). After preliminary tests (data not showed) with product development team was possible to obtain the formulations to be evaluated sensorially.

Sensory analysis

Quantitative descriptive analysis (QDA)

The sensory profile of each energy gel sample was determined using the QDA. First, it was performed recruitment and pre-selection of panelists. The panelists were recruited from students and staff of FURG who had already been screened in previous sensory teams for their performance on odor recognition and basic tastes (ASTM, 1981). For the initial survey of terms, 3 samples representing marked differences in aroma, flavor and texture attributes were chosen. The samples were presented simultaneously and the panelists were asked to describe the similarities and the differences between them (Moskowitz, 1983).

Thus, it was elaborated an evaluation form containing the descriptive terms chosen in consensus by the sensorial team. In the form it was used an unstructured scale of 9 cm, anchored at the extremes by "none" or "weak" to the left and "strong" to the right.

In the final selection of the team, three samples were evaluated in a randomized complete block design with two replicates. The candidates were selected based on the ability of discrimination between samples, repeatability and agreement between the panelists in order to form a homogeneous team (Damásio and Costell, 1991).

To evaluate the samples, these were presented to the panelists of monadic manner. The data were evaluated using an analysis of variance (ANOVA) with 90% confidence, and the mean difference was obtained by the Tukey test. The samples A, A_1 , A_2 , and B were used in the analysis.

Acceptance sensory and purchase intent

The panel of assessors to acceptance test and purchase intent test was composed for 110 individuals, practitioners of physical activities, of both genders, between 14 and 65 years old. To evaluate the acceptance of energy gel samples of banana with acai berry flavor, samples were presented to the panelists in plastic cups coded with threedigit numbers. The presentation of the samples was performed monadic manner and asked to panelists to rate the samples globally using hedonic scale of nine points ranging from (9) "extremely liked" to (1) "extremely disliked". The acceptance rate was calculated according to Queiroz and Treptow (2006). The five-point scale ranging between (5) "certainly will buy and" (1) "certainly won't buy" was used to evaluate the purchase intent (Meilgaard et al., 1999). The formulations A_1 and A_2 were evaluated in acceptance test and purchase intent.

Along the sensory analysis was carried out a questionnaire to evaluate the consumption of food supplements by the panelists and their familiarity with energy replenishers gel.

Results and Discussion

Quantitative descriptive analysis (QDA)

The terms selected to compose the selection of records of panelists and evaluations of the samples were for aroma attribute: artificial acai berry and artificial banana; for flavor attribute: artificial acai berry, artificial banana and *Spirulina*; for texture attribute: viscosity and adhesiveness.

Based on the analysis of variance (ANOVA), four panelists were eliminated and nine panelists were selected to participate in the evaluation of the samples. The results of the scores awarded by the selected panelists for each descriptive term of the four samples is shown in Table 1.

The descriptive terms "acai berry aroma" and "acai berry flavor" showed no significant difference between samples. With regard to terms olfactory sensations, the B sample was characterized by "banana flavor", differing from the A_1 and A_2 samples. Probably the *Spirulina* may have covered up the "banana flavor", which was not observed for "acai berry flavor". The A and A_1 samples showed similar profiles. Although the A_2 formulation has the highest concentration of *Spirulina*, it presented as a predominant characteristic texture attributes.

According about the viscosity and adhesiveness

Table 1. Values attributed to the descriptive terms of the samples

	F			
Descriptive term	Samples			
	Α	A 1	A ₂	В
Acai berry aroma	2.54ª	2.63ª	1.73ª	2.33ª
Banana aroma	2.57 ^{ab}	1.26ª	1.69 ^{ab}	3.39 ^b
Acai berry flavor	3.34ª	3.33ª	2.30ª	2.30ª
Banana flavor	2.51 ^{ab}	1.57ª	1.62ª	3.62 ^b
Spirulina flavor	0.24ª	1.79 ^b	2.57 ^b	0.00ª
Viscosity	4.41 ^{ab}	4.09 ^{ab}	5.37⁵	5.85ª
Adhesiveness	4.42 ^{ab}	3.81 ^{ab}	5.58 ⁵	1.78ª

Legend: A - without addition of *Spirulina* and 0.25% (w/w) pectin; A1 - 0.35% (w/w) *Spirulina* and 0.25% (w/w) pectin; A2 - 0.50% (w/w) *Spirulina* and 0.50% (w/w) pectin; B - Commercial sample (without *Spirulina* in its composition). Results with the same letters in the same line indicate no significant difference at a 90% confidence level by the Tukey test.

terms, it can be observed the increased of both with the raise the concentration of the pectin on the gel development. This was also observed by Mesbahi *et al.* (2005), in a comparative study on functional properties of beet and citrus pectins in food systems. Pectin is responsible for the formation of threedimensional gel network to form links to each other through hydrogen bonding. To the gel formation is necessary reduce the bonds between the water molecules and the molecules of pectin and facilitate the attraction of the pectin molecules (Morris, 2007). The viscosity of a pectin solution is related to its physical and chemical characteristics (degree of esterification, molecular mass) and concentration (Mesbahi *et al.*, 2005; Villay *et al.*, 2012).

Based on the average values of the scores assigned by the panelists was prepared, for each descriptive term, the sensory profile of energy gel samples of banana with Brazilian berry flavor. The commercial sample excelled in the terms viscosity, banana flavor and aroma, compared to the gel developed, while this latter, highlighted in relation to the adhesiveness. In relation to the aroma and flavor of acai berry, as previously reported, there was no significant difference, which can be confirmed by viewing the Figure 1.

Acceptance and purchase intent tests

Regarding the sensory analysis, average acceptance rates were 6.7 ± 1.65 and 6.6 ± 1.78 for samples A₁ and A₂, respectively. Both samples (A₁ and A₂) were accepted by the target public (acceptance rate of 74%). Carvalho *et al.* (2016) developed food for athletes (electrolyte replenisher, muscle recovery

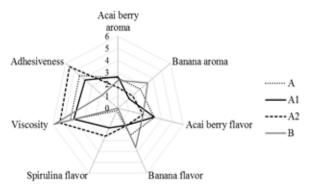


Figure 1. Sensory profile of developed energy gels: A - without addition of *Spirulina* and 0.25% (w/w) pectin; A1 - 0.35% (w/w) *Spirulina* and 0.25% (w/w) pectin; A2 - 0.50% (w/w) *Spirulina* and 0.50% (w/w) pectin; B - Commercial sample (without *Spirulina* in its composition).

supplement and muscle enhancer) and found that all developed products with 0.50% (w/w) of *Spirulina* were accepted by the target public with acceptance above 70%.

With regard to purchase intent, it was observed that 38.5% of the judges "probably would buy" the product added of 0.35% (w/w) of *Spirulina* (formulation A₁). For product added of 0.50% (w/w) of *Spirulina* (formulation A₂), 50.5% of panelists had opinion between "probably" and "certainly would buy" and only 3.7% chose "certainly would not buy" (Figure 2).

Probably the purchase intent for energy gel with *Spirulina* is because the *Spirulina* powder it is innovative and attractive. In addition to its nutritional role, *Spirulina* serves as a good source of protein and natural coloring agent (Dey and Rathod, 2013; Zheng *et al.*, 2017). These factors and the characteristic flavor of microalgal biomass may have contributed to the sensorial results obtained.

Spirulina has received increasing attention due to the fact that it is source of compounds with biological activity. Other studies present results that demonstrate consumers' interest in foods with this microalga. Morais et al. (2006) developed cookies without Spirulina (control) and cookies containing 1.0; 3.0 and 5.0% (w/w) of dry biomass of Spirulina and found that 58 and 50% of the panelists would buy cookies with 1.0 and 5.0% (w/w) Spirulina, respectively. Moreover, they proved that the samples containing 1.0 and 3.0% (w/w) of Spirulina did not differ significantly of control cookie regarding appearance. Concerning color, the judges also found no difference between the four samples. In study of Carvalho et al. (2016), the athletes also had positive attitudes towards the purchase of the products with microalgal biomass. Thus, Spirulina biomass can be added in foods for athletes and practitioners of

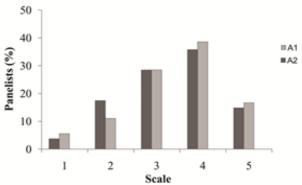


Figure 2. Purchase intent of energy gel: A1 (0.35% (w/w) *Spirulina* and 0.25% (w/w) pectin) and A2 (0.50% (w/w) *Spirulina and* 0.50% (w/w) pectin). (1 - Certainly would not buy; 2 - Probably would not buy; 3 – In doubt whether would buy or not; 4 - Probably would buy; 5 – Certainly would buy).

physical activities without to influence the sensory opinion of consumers.

Nutritional information

According to ANVISA (2010), energy supplements should contain at least 15.0 g of carbohydrates per serving of the product ready for consumption. Carbohydrates are an important energy source for the metabolism of athletes and the developed products provide this calorie intake (Table 2).

The low-carbohydrate diet quickly commits glycogen reserves for both rigorous physical activity and for regular training. Thus, the consumption of energy gel contributes to avoiding the risk of hypoglycemia, improving yield and effectiveness of athletes (Burke *et al.*, 2011).

The effect of using carbohydrate gel for the improvement in terms of performance lacks knowledge in the literature. However, according to Hernandez and Nahas (2009), the use of energy gel during exercise fulfills the role in carbohydrate replacement, though its use should be accompanied by regular intake of water, so that its combination ensures the maintenance of the performance of an organism properly hydrated.

The energy gel developed with *Spirulina* provides protein and carbohydrate that may increase insulin and decrease cortisol levels and reduce muscle damage by an average of 27% and muscle soreness by 30% in runners (Spiller *et al.*, 1987; Luden *et al.*, 2006). Thus, *Spirulina* can improve the functional properties of the product due to its high content of proteins and carbohydrates.

The increased in oxygen consumption during or after exercise results in the formation of oxygen free radicals. Thus, the addition of *Spirulina* in

Table 2. Nutrition information of energy gel added	of
Spirulina (serving size 1 packet - 32 g)	

Nutrition information	Formulation A ₁	Formulation A ₂	
Energy (kcal)	87.52 87.52		
Carbohydrate (g)	21.78	21.73	
Protein (g)	0.10	0.14	
Total Fat (g)	0.00	0.00	
Saturated Fat (g)	0.00	0.00	
Trans Fat (g)	0.00	0.00	
Cholesterol (mg)	0.00	0.00	
Dietary Fiber (g)	0.00	0.00	
Calcium (mg)	8.10	8.08	
Iron (mg)	0.70	0.70	
Sodium (mg)	72.07	72.04	
Potassium (mg)	130.67	130.67	
Magnesium (mg)	40.85	40.85	

Legend: A1 - 0.35% (w/w) *Spirulina* and 0.25% (w/w) pectin; A2 - 0.50% (w/w) *Spirulina* and 0.50% (w/w) pectin.

foods for athletes is interesting because the *Spirulina* biomass has carotenoid, phycocyanin, and phenolic compounds, which are known for their antioxidant activity (Vaz *et al.*, 2016).

Spirulina and moderate exercise showed decreased levels of a marker of oxidative stress in the brain and serum (Thiobarbituric Acid Reactive Substances) (Mazzola *et al.*, 2015). Moreover, the benefits of *Spirulina* supplementation induced a significant increase in exercise performance, fat oxidation, and glutathione concentration and attenuated the exercise-induced increase in lipid peroxidation (Kalafati *et al.*, 2010).

Based on the panelists responses for questionnaire performed along with the acceptance and purchase intent test, it was observed that approximately 39% had consumed some type of dietary supplement and 67% of them claimed to use dietary supplement regularly. Among the participants who claimed that regularly use a dietary supplement, 51% were women. When asked about the consumption of energy gels, only 6.5% of the judges had already experienced this type of product which being predominantly man and age group between 20 and 40 years old. The low percentage of assessors who had experienced some energy gel may have influenced the scores assigned by the judges to the elaborate formulations.

Conclusion

The present study showed a sensory profile for

energy replenisher gel added of *Spirulina*. The terms relevant to description of the product quality were "artificial acai berry" and "artificial banana" for aroma attribute; "artificial acai berry", "artificial banana" and "*Spirulina*" for flavor attribute; "viscosity" and "adhesiveness" to the texture attribute. The products developed with *Spirulina* were accepted (74%) by the target public. Moreover, positive attitudes were found to the purchase of the enriched energy gels with microalgal biomass. Therefore, *Spirulina* biomass may be added in energy gels destined for practitioners of physical activities without affecting the sensory characteristics of the products.

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