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A N I N T E R N A T I O N A L J O U R N A L

Novel Food Supplements Formulated With S*pirulina* To Meet Athletes' Needs

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ABSTRACT

The food, training, and health are crucial for a good performance in sports. Intense physical activity takes the athlete to maintain a very unstable balance between energy demand and consumption of nutrients. Spirulina microalga has a nutritional profile that renders it an ideal food supplement, because has high protein content, also contains vitamins, minerals, and pigments. In this context, the study aimed to develop, characterize and evaluate the stability of foods enhanced with Spirulina, which are intended for athletes. In this study, six different supplements were developed (electrolyte replenisher, muscle enhancer, and recovery supplement), without and with Spirulina. The electrolyte replenisher with Spirulina compared to the product without the microalga, showed an increase of 0.35% (w/w) in mineral content. The carbohydrates content of the developed recovery supplement with Spirulina was 2% (w/w) higher than the muscle enhancer without Spirulina. It was not observed increased in the nutritional content of muscle recovery when added Spirulina. However, it is known that Spirulina presents active compounds with important functions for the body. Thus, the composition of the foods satisfied the nutritional needs of athletes. Regarding the stability of developed foods, the shelf life was estimated between 9 and 11 months.

Key words: athletes; microalgal biomass; performance; supplement.

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INTRODUCTION

An appropriate energy intake is important in athlete's diet because determines the capacity for intake of macronutrient and micronutrients, and assists in manipulating body composition[1]. Generally, protein intake by athletes tends to be higher than recommended[2], while that consumption of carbohydrates is sometimes below the recommended range[3]. The adequate consumption of some micronutrients is also a source of concern, with some studies showing intakes under the dietary reference intakes (DRI). Sports nutrition guidelines suggest that protein intake should be between 1.2 and 1.7 g/kg/d, carbohydrates ingestion may range from 3.0 to 12.0 g/kg/d depending on the duration and type of exercise, and fat should contribute to 20-35% of total energy value. Moreover, athletes should reach, at least, DRI for all micronutrients[4,5].

The feeding of the athletes is distinguished from other individuals due to the high-energy consumption, which varies according to the type of activity and training. Carbohydrates are an important energy source for the metabolism of humans. Athletes need a carbohydrate intake higher than non-athletes. A low-carbohydrate diet quickly commits glycogen reserves for both rigorous physical activity and for regular training. With poor carbohydrate diet, besides the risk of hypoglycemia, athletes may be affected in terms of yield and effectiveness. The high-intensity training will promote greater uptake of glucose by the active muscle, which will be metabolized anaerobically. Thus, the lactate concentrations increase and plasma pH decrease, causing discomfort in athletes[5,6-8.]

Proteins are essential components of all cells of the body and helps in maintain the immune system. The protein requirements of athletes are frequently discussed due to an increased demand from exercise and the benefits of amino acid supplementation on performance. Due to its satiating efficiency, protein consumption has generally been favored for its role in controlling food intake to favor weight loss and prevent weight gain in athlete and non-athlete groups[9]. In addition, the combined ingestion of protein and carbohydrate improves bench press performance and increases blood glucose levels[10]. Protein added to carbohydrate may increase insulin; decrease cortisol levels, and reduce muscle damage by an average of 27% and muscle soreness by 30% in runners[11,12].

Hydration of athletes also is important, since without fluid replacement performance decreases significantly. In certain cases, the water consumption is not sufficiently effective, making necessary the replacement of minerals[13]. The supplementation of minerals and vitamins may be required to cover increased needs for building, repair, and maintenance of lean body mass in athletes[14]. Many of the B-complex vitamins are essential to help form hemoglobin in red blood cells, a major determinant of oxygen delivery to the muscles during aerobic endurance exercise. The vitamins C and E present important functions for preventing oxidative damage to cellular and subcellular structure and function during exercise training, optimizing the preparation for competition[15].

In this context, the supplements are marketed to meet the energy and nutritional demands of athletes. Electrolyte replenishers are classified as electrolyte supplements and are products designed to aid hydration of athletes, replenishing water and minerals removed by transpiration during physical activities. Muscle enhancers are classified as creatine supplements and are used by athletes to complement the endogenous stocks of creatine. The muscle enhancers may be added carbohydrates, however, it can not be added to dietary fiber. Energy supplements as muscle recovery, supplement the energy needs of athletes. This product ready for consumption should contain a minimum of 75% of total energy intake from carbohydrates[16]. Thus, it is important to study the development of these products

because they are able to improve performance, increase muscle mass, help control or lose weight, prevent illness and disease, boost immunity, and reduce stress in athletes[17].

Microalgae represent a new and promising potential source of a vast number of natural bioactive compounds for functional food development[18]. *Spirulina* microalga has the advantage of being safe for use in foods (Generally Recognized as Safe - GRAS)[19]. *Spirulina* is also useful as a functional ingredient because the biomass can be incorporated in various food products to enhance nutritional quality and for therapeutic action on chronic diseases[20,21]. This microalga has a high protein content and the amino acids in *Spirulina* are similar to the amino acids recommended by the Food and Agricultural Organization. *Spirulina* biomass also contains vitamins, minerals, and pigments[22-24]. This study aimed to develop, characterize and evaluate the stability of foods enhanced with *Spirulina*, which are intended for athletes.

MATERIAL AND METHODS

Biomass of *Spirulina* Microalga

The microalga added to foods was S*pirulina* sp. LEB 18, 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[25]. The *Spirulina* biomass was subjected to operations of drying, grinding in a ball mill (Model Q298, Quimis, Brazil) and sieving to obtain particles with a diameter of 0.053 mm. Subsequently, it was vacuum packed for use in formulations.

Development of Formulations

Six foods were developed for athletes: an electrolyte replenisher, a muscle recovery supplement and a muscle enhancer. The following ingredients were used to prepare the electrolyte replenisher: sucrose, dextrose, citric acid, tricalcium phosphate, sodium citrate, sodium chloride, potassium phosphate antiwetting agent, nature-identical aroma of lemon, sucralose sweetener and titanium dioxide.

The final formulation of the muscle enhancer included maltodextrin, dextrose, creatine, nature-identical aroma of lemon, citric acid, tricalcium phosphate antiwetting agent, acesulfame potassium and sucralose sweeteners and titanium dioxide.

The muscle recovery supplement formulation contained the following ingredients: maltodextrin, milk whey protein concentrate 80% (w/w), dextrose, vitamin and mineral premix [magnesium sulphate monohydrate (magnesium), ascorbic acid (vitamin C), ethyl tocopherol (vitamin E) and zinc sulphate monohydrate (zinc)], medium chain triglycerides, nature-identical aroma of lemon, guar gum thickener, citric acid, acesulfame potassium and sucralose sweeteners.

The ingredients were weighed and homogenized using a Y-type mixer (Model TE-201/10, Tecnal, Brazil). The foods were produced with (0.5%, w/w) and without the addition of *Spirulina*. All products were developed following legislation of Brazilian National Health Surveillance Agency (ANVISA)[16], as well as similar products available on the market. The concentration of *Spirulina* also was defined based on the homogenization of the final product (data not shown) and the legislation of ANVISA. According to ANVISA, the daily recommendation of product consumption should not result in *Spirulina* intake above 1.6 g[26].

Chemical Composition

Moisture, ashes, protein and lipids contents were determined according to the AOAC[27]. The carbohydrate content was measured using an adaptation of the DNS method[28], with a prior acid hydrolysis of polysaccharides. The results were analyzed using ANOVA with a 95% confidence interval and the differences of means were tested using Tukey's test.

Shelf Life of Developed Foods

The shelf life of each product was estimated using a mathematical model that relates the increase in product moisture determined by a moisture sorption isotherm to the water vapor transmission rate (WVTR) of the packaging. This phenomenon can be described by Equation 1[29], where *t* is the estimated shelf life (days); *Md* is the dry mass of the product (g); *RH* is the relative humidity of the storage environment (%, w/w); *A* is the packaging area (m^2); *WVTR* is the water vapor transmission rate of the packaging ($g_{water}.day^{-1}$); aw (M) is the water activity of the product according to the moisture content, i.e., from the sorption isotherm of the moisture product; *Mi* is the initial moisture of the product (%, w/w); and *Mc* is the critical moisture of the product (%, w/w).

$$t = \frac{Md.RH}{100.A.WVTR}\int_{Mi}^{Mc}\frac{dM}{\frac{RH}{100} - aw(M)} \quad (1)$$

The WVTR ($g_{water}.m^{-2}.day^{-1}$) was determined by a gravimetric method using the methods described in ASTM E96/E96-05[30]. Transmission was evaluated using high-density polyethylene (HDPE) packaging that was sealed with a plastic screw cap and a jar with a thickness of 1 mm, a height of 57.2 mm, a diameter of 35.5 mm, and a capacity of 30 mL.

The sample was weighed in triplicate, stored in HDPE packages without covers and conditioned in desiccators that contained saturated salt solutions, with a relative humidity range between 22 and 90%. The desiccators were maintained in an incubator at 30 °C until the moisture gain of the samples stabilized. After this period, the equilibrium moisture content was determined for each relative humidity condition. The critical moisture of the products was established based on the changes that occurred during storage under different relative humidity conditions. The initial and critical moisture was measured according to the AOAC [27].

The experimental data from the sorption isotherms of the products were fit by the Halsey equation (Equation 2)[31], where *aw* is the water activity of the product, *M* is the moisture of the product (%, w/w), and C_1 and C_2 are constants.

$$aw = \exp\left(-C_1/M^{C_2}\right) \quad (2)$$

RESULTS AND DISCUSSION

Chemical Composition

The electrolyte replenisher was a product intended to assist hydration, and according to its chemical composition, the developed drinks can be utilized to replenish the water and minerals removed by sweating during physical activities. An indication of the consumption of electrolyte replenisher was 40.0 g in 500 mL of water with intake before, during and/or after physical activity, which providing 36.5 g of carbohydrate in the product with *Spirulina* (Table 1). According to BRASIL[16], among the main

characteristics of this type of supplement, carbohydrates can be up to 8% (w/v) of the product ready for consumption. In addition, the concentration of sodium in the product ready for consumption should be between 460 and 1150 mg/L and the product may be added potassium up to 700 mg/L. The developed replenishers present 7.4% (w/v) of carbohydrates, 512 mg/L of sodium and 68 mg/L of potassium in the final product for consumption.

Table 1 - Chemical composition of the developed electrolyte replenishers.

Composition	Replenisher with *Spirulina* (%, w/w)	Replenisher without *Spirulina* (%, w/w)
Carbohydrates*	91.14 ± 1.99^{a}	92.68 ± 0.81^{a}
Ashes*	4.00 ± 0.06^{b}	3.65 ± 0.06^{c}
Lipids*	0.22 ± 0.02^{d}	0.17 ± 0.00^{d}
Moisture*	2.11 ± 0.04^{e}	2.82 ± 0.02^{e}
Sodium**	0.64	0.64
Potassium**	0.17	0.17

Same letters in the same line indicate that means do not differ significantly ($p > 0.05$) using the Tukey test.
* Results are the means of the three measurements on a dry basis ± standard deviation.
** Results according to formulation.

We also were able to verify that the products with and without *Spirulina* showed low moisture contents and low lipid contents based on the characteristics of the product (which is a carbohydrate replacement). The ash content presented in electrolyte replenisher with *Spirulina* was higher than the product without microalgal biomass, probably due to the minerals present in this microalga, since the developed products had the same concentration of added salts. The ashes represent the content of phosphates, potassium, sodium and other salts that were added to the products. The incorporation of *Spirulina* in the electrolyte replenisher has some advantages for the improved hydration and performance of athletes, particularly in the replacement of minerals. According to Branger et al.[32] and Henrikson[33], the minerals present in greater quantities in *Spirulina* are calcium, iron, phosphorus, magnesium, manganese, potassium and zinc, and the main ones are calcium (1.3 to 1.4 g/kg *Spirulina*), phosphorus (from 6.7 to 9.0 g/kg *Spirulina*) and potassium (6.4 to 15.4 g/kg *Spirulina*).

In respect of the developed muscle enhancers, the addition of *Spirulina* increased the carbohydrate content in the product (Table 2). These products are primarily aimed at increasing the performance of athletes by increasing muscle creatine and carbohydrate intake. The enhancer had an indication of consumption of 6.0 g in 200 mL of water with an intake 30 min prior to training, which indicates 1.6 g of creatine in the products. According to BRASIL[16], the product ready for consumption should contain 1.5 to 3.0 g of creatine in the portion.

Table 2 – Chemical composition of the developed muscle enhancers.

Composition	Enhancer with *Spirulina* (%, w/w)	Enhancer without *Spirulina* (%, w/w)
Carbohydrates*	63.33 ± 0.26^{a}	61.33 ± 0.07^{b}
Ashes*	1.15 ± 0.00^{c}	1.12 ± 0.00^{c}
Lipids*	0.07 ± 0.01^{d}	0.08 ± 0.01^{d}
Moisture*	8.50 ± 0.01^{e}	9.16 ± 0.02^{e}
Creatine**	26.83	28.16

Same letters in the same line indicate that means do not differ significantly ($p > 0.05$) using the Tukey test.
* Results are the means of the three measurements on a dry basis ± standard deviation.
** Results according to formulation.

The compounds that exist in higher quantities in the formulated muscle enhancers are creatine and carbohydrates. This type of product is intended to complement the endogenous creatine stock. According to Green et al.[34], the combination of creatine with carbohydrates causes the muscle creatine stores to easily attain their maximum levels. The authors demonstrated that the combination of creatine with a simple carbohydrate, such as glucose, increases creatine transport within the muscle, even in individuals with almost normal muscle creatine levels. Breen et al.[35] have shown that myofibrillar protein synthesis increases when protein is co-ingested with carbohydrates after cycling exercise. Furthermore, the addition of *Spirulina* is an alternative to the increment of some minerals in the product, especially phosphorus which participates in the resynthesis of phosphocreatine in the muscle and is present in greater quantity in the microalgae[32].

The muscle recovery supplements are a source of carbohydrates (Table 3). According to BRASIL[16], this type of product was intended to complement the needs energy of the athletes and the quantity of carbohydrate in the energy supplements should be at least 15.0 g portion of the product ready for consumption. The product with microalga contains 30.3 g of carbohydrates; as an indication of muscle recovery consumption, a portion was defined as 40.0 g of product in 350 mL of water with intake during or after exercise. One of the main advantages of carbohydrate intake during exercise is the maintenance of glycemia that allows blood glucose to sustain the energy demand of muscles for a long period, reducing the rate of glycogen depletion[6]. The two recovery supplements contained the same amount of lipids, which were incorporated into the formulation to spare muscle and liver glycogen and improve performance during prolonged exercise.

Table 3 - Chemical composition of the developed muscle recovery supplements.

Composition	Recovery supplement with *Spirulina* (%, w/w)	Recovery supplement without *Spirulina* (%, w/w)
Carbohydrates*	75.85 ± 0.42^{a}	82.28 ± 0.18^{b}
Proteins*	17.42 ± 0.10^{b}	17.28 ± 0.09^{b}
Lipids*	2.13 ± 0.02^{c}	2.13 ± 0.03^{c}
Ashes*	1.68 ± 0.01^{e}	1.85 ± 0.00^{d}
Moisture*	6.77 ± 0.14^{f}	6.85 ± 0.10^{f}

Same letters in the same line indicate that means do not differ significantly ($p > 0.05$) using the Tukey test.
*Results are the means of the three measurements on a dry basis ± standard deviation.

The developed foods were specially formulated to help athletes meet their specific nutritional needs and assist in the performance of exercise with intense muscular effort. Though the concentration of the microalgae used in muscle enhancer has not contributed in checking the nutritional increase, as well as some components of other products (replenisher, muscle enhancers), the addition of *Spirulina* in these types of foods for athletes can provide benefits in terms of functional properties. According to Oliveira et al.[36] and Belay[23], the development of foods with addition of *Spirulina* may be interesting and beneficial, because this microalga contains antioxidants, vitamins, minerals, and essential fatty acids, has a protein content of approximately 60-70% (w/w) and contains all essential amino acids.

The athletes are more susceptible to oxidative reactions that produce more free radicals when they practice intense and prolonged physical activities. The increased oxygen consumption, as well as the activation of specific metabolic pathways during or after exercise, results in the formation of oxygen free radicals. The free radicals are related to a number of diseases such as pulmonary emphysema, inflammatory diseases, atherosclerosis, cancer and aging[37-41]. In addition, an inadequate antioxidant

defense system may increase oxidative stress, which is accompanied by metabolic changes, and leads to poor performance in athletes[42].

Spirulina biomass contains carotenoid, phycocyanin, and phenolic compounds, which are known for their antioxidant activity[43-47]. The phycocyanin and allophycocyanin are the pigments that can be found in thylakoids of cyanobacteria, such as *Spirulina*, and are studied due to their ability to react with reactive substances generated during the oxidative process[48]. Bertolin et al.[49] showed that the antioxidant potential of the phycocyanin present in *Spirulina* and indicated that the amount administered (5mg/d) was sufficient to reduce the oxidative damage caused by monosodium glutamate in vivo. According to Mazzolla et al.[50], *Spirulina* and moderate exercise showed decreased levels of Thiobarbituric Acid Reactive Substances (TBARs), a marker of oxidative stress in the brain and serum. This combination also prevented an increase in serum cholesterol and decreased triglycerides (TG) levels. Therefore, moderate exercise and *Spirulina* might be useful in treating the cardiovascular, neurodegenerative and other diseases related to aging. Kalafati et al.[51] reported the benefits of *Spirulina* combined with physical exercise in humans. *Spirulina* supplementation induced a significant increase in exercise performance, fat oxidation, and glutathione (GSH) concentration and attenuated the exercise-induced increase in lipid peroxidation.

In development of novel foods, the sensory analysis contributes to the quality and acceptance of these products. In a previous study[52], it was shown that the products developed (replenisher, muscle enhancer, and recovery supplement) added of *Spirulina* are accepted by the target public (athletes) with acceptance above 70%. The previous study showed that athletes have positive attitudes towards the purchase of these products with *Spirulina* biomass. Thus, 0.5% (w/w) of *Spirulina* can be added to foods for athletes without to influence the opinion of consumers likely. Moreover, due to its functional properties, *Spirulina* can be considered a differential in the market for food supplements, contributing to the good performance of the athletes.

Shelf Life of Developed Foods

Regarding the stability of the developed foods, the WVTRs of the food packed in HDPE with an area of 6.38×10^{-3} m^2 and a thickness of 1 mm were evaluated over 22 days. The WVTR for this packaging was obtained from the slope ratio (0.0052) of the equation of the line and the area of the package, which provided a value of 0.8 $g_{H2O}.m^{-2}.day^{-1}$ at 90% relative humidity and 30°C.

With respect to the isotherms, the developed food with and without *Spirulina* showed similar behaviors. Based on the changes observed in the samples, the critical moisture was set to a 0.32 water activity with 3.00% (w/w) moisture for the replenisher without *Spirulina* and 2.30% (w/w) moisture for the replenisher with the microalga. The critical moisture of the enhancer was set to 9.48% (w/w) for the product without *Spirulina* and 8.82% (w/w) for the product with the microalga; they had a water activity of 0.43. For the same water activity, the critical moisture levels of 7.14% and 7.10% (w/w) were defined for the recovery supplement without *Spirulina* and with *Spirulina*, respectively.

The correlation coefficient (r) indicates the quality of the fit. Equation 2 provided a good fit for the experimental data from the isotherms of the developed foods (Table 4). Studies were performed with mathematical models to estimate the shelf life of food[53,54]. Amodio et al.[55] assessed the shelf life of fresh rocket using the Weibullian model to fit the experimental data. The authors obtained correlation coefficients

between 0.95 and 0.99. Thus, the shelf life of foods with *Spirulina* can also be demonstrated with the use of mathematical models.

Table 4 - Experimental data from the sorption isotherms of the products, which were fit by the Halsey equation.

Sample	C_1*	C_2*	r
Replenisher without *Spirulina*	1.83 ± 0.12	0.38 ± 0.03	0.98
Replenisher with *Spirulina*	1.58 ± 0.06	0.32 ± 0.02	0.99
Enhancer without *Spirulina*	41.98 ± 13.09	1.69 ± 0.13	0.99
Enhancer with *Spirulina*	19.60 ± 6.48	1.41 ± 0.14	0.98
Recovery supplement without *Spirulina*	14.01 ± 3.05	1.41 ± 0.10	0.99
Recovery supplement with *Spirulina*	12.36 ± 1.71	1.34 ± 0.06	0.99

*C_1 and C_2 obtained by Equation 2.

To measure the shelf life of the foods developed with and without the addition of *Spirulina*, the Halsey Equation 2 was replaced in Equation 1. The electrolyte replenishers with *Spirulina* and without *Spirulina* showed an initial moisture content of 2.11% and 2.82% (w/w), respectively. Therefore, a shelf life of 9 months was estimated.

Regarding the enhancers with *Spirulina* and without *Spirulina*, which showed an initial moisture content of 8.50% and 9.16% (w/w), respectively, the enhancer with *Spirulina* had a 9 month shelf life, whereas the enhancer without *Spirulina* had a shelf life of 11 months. The muscle recovery supplement developed with *Spirulina* and without *Spirulina* showed an initial moisture content of 6.77% and 6.85% (w/w), respectively. The food without *Spirulina* obtained an estimated shelf life of 9 months, whereas the product with the microalga had an estimated shelf life of 11 months. This difference may be attributed to a greater adsorption of water by the foods with *Spirulina* or the significant difference between the critical moisture content and the initial moisture content in the products without *Spirulina*.

CONCLUSION

The addition of *Spirulina* contributed for increased of some compounds that are important for improving the performance of athletes. The mineral content of the electrolyte replenisher with *Spirulina* was higher than the product without addition of microalga. Furthermore, *Spirulina* biomass afforded increase in the carbohydrate content of muscle enhancer developed. Regarding muscle recovery added *Spirulina*, it was not observed increased in the product composition in nutritional terms. However, it is known that *Spirulina* presents active compounds with important functions for the body, with the potential to the improved performance of athletes. Thus, it is important to perform further scientific studies that can demonstrate and clarify the functional benefits of these products added *Spirulina*, for the athletes. About the shelf life for the developed foods, the period was estimated between 9 and 11 months, and it was according with similar commercial products.

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