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Novel foods: a meal replacement shake and a high-calorie food supplemented with *Spirulina* biomass

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Abstract

Spirulina (*Arthrospira*) is a microalga that has high protein content and is rich in minerals and vitamins; characteristics that make it a food additive for different population groups. The present work was aimed to develop, characterise and evaluate the stability and acceptance of a meal replacement shake and a high-calorie food, both with *Spirulina* sp. LEB 18. The average carbohydrate and protein contents for high-calorie food supplement were 71% and 17%, respectively. The meal replacement shakes had 85% of carbohydrates and 19% of protein on average. According to sensory test, the target group accepted both foods developed with *Spirulina* sp. LEB 18. The shelf life of the products with microalgal biomass was estimated at 26 months for the high-calorie food supplement and 17 months for the meal replacement shake. Thus, the biomass of *Spirulina* sp. LEB 18 can be considered an interesting alternative for the nutritional intake.

Introduction

Many athletes consume nutritional supplements to maximise the results of their physical training. When carbohydrates and amino acids are consumed before exercise, protein synthesis is higher compared to consumption after exercise (Tipton *et al*., 2001). The intake of supplements containing carbohydrates and proteins provides additional fuel for the athlete, while increasing the levels of blood glucose and insulin (Bird *et al*., 2006).

Overweight and obesity, as well as their association with other diseases cause and aggravate many health problems. The increase in obesity in recent years makes prevention a global priority. These factors have stimulated the intake of different foods, which are able to meet the daily nutritional needs (Galli and Reel, 2009; Pope *et al*., 2015). The growing number of men and women who seek to reduce levels of body fat and increase muscle mass justify the development of products such as high-calorie foods and meal replacement shakes.

Microalgae (e.g., *Spirulina*, *Chlorella*) represent innovative alternative natural ingredients that can be used in the development of healthy foods. *Spirulina* is a source of proteins and rich in amino acids, phytonutrients, antioxidants, carbohydrates, mucopolysaccharides, vitamins and minerals, and beyond the health benefits, the developed products present appealing and stable colours. The biomass of these microalgae helps speed up metabolism, preventing fat storage (Batista *et al*., 2012; El-Tantawy *et al*., 2015).

Considering all these characteristics of *Spirulina*, it can be considered a dietary supplement for athletes. The consumption of *Spirulina* has shown results such as protective effect from exercise-induced muscle damage, a decrease in lactate dehydrogenase level in the blood, inducement of blood superoxide dismutase activity after endurance exercise, and an increase in glutathione peroxidase level in the blood (Lu *et al*., 2006). For female athletes, an increase in their haemachrome level was confirmed after taking 10 g *Spirulina* pills per day for four weeks (Li and Qi, 1997). Also, the addition of *Spirulina* in the diet has effects in lowering the serum levels of glucose, cholesterol and triglycerides (Hosoyamada *et al*., 1991; Belay *et al*., 1993; Ramamoorthy and Premakumari, 1996; Walker *et al*., 2005).

Furthermore, *Spirulina* is certified as GRAS (Generally Recognized as Safe) and can be consumed without posing a health risk (FDA, 2012). Therefore,

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this microalga can be marketed as a food supplement and/or used as an ingredient in food formulations. Foods like meal replacement shake and high-calorie food, which are mainly consumed by athletes, are not yet marketed with microalgal biomass supplementation. Given the above, the objective of the present work was to develop, characterise and evaluate the stability and acceptance of meal replacement shake and a high-calorie food with the addition of *Spirulina* sp. LEB 18.

Materials and methods

Food supplement development

The ingredients of the developed foods, a meal replacement shake, and a high-calorie food supplement, were selected based on the specific nutritional needs of each population group. For the preparation of formulations, preliminary tests were performed varying the proportions of the components.

The formulation of the high-calorie food supplement was composed of whey protein isolate (WPI), whey protein hydrolysate (WPH) and whey protein concentrate (WPC), collagen, maltodextrin, glucose, medium chain triglycerides (MCT), chocolate flavour, acesulfame potassium, cocoa and *Spirulina* sp. LEB 18 (750 mg/100 g).

For the meal replacement shake, the selected ingredients were maltodextrin, WPC, concentrated soy protein, oatmeal, collagen, polydextrose, xanthan and guar gums, natural flavour, cocoa, tricalcium phosphate, acesulfame potassium, sucralose, caramel colouring, mix of minerals and vitamins (vitamins A, D, B_1, B_2, PP, B_5, B_6, B_{12}, C, E, H and K, folic acid, iron, zinc, copper, iodine, selenium, manganese, fluoride, molybdenum, chromium, magnesium, potassium and sodium) and *Spirulina* sp. LEB 18 (750 mg/100 g).

For the development of the food supplement, the ingredients were weighed (Model F4 2104N, Bioprecisa, Brazil) and mixed in a type Y mixer (Model TE-201/10, Tecnal, Brazil). The biomass of *Spirulina* sp. LEB 18 was produced in the pilot plant of the Laboratory of Biochemical Engineering, located near the shore of Mangueira Lagoon (33° 30’ 13’’S and 53° 08’ 59’’ W) in the city of Santa Vitória do Palmar, Brazil (Morais *et al*., 2009). The *Spirulina* sp. LEB 18 biomass was dried at 50°C for 5 h in a tray-dryer, and then vacuum-packed and stored. For better solubilisation, the dry biomass was ground in a ball mill (Model Q298, Quimis, Brazil) and sieved with a granulometric Sieve Shaker (Bertel, Brazil) with a 270-mesh sieve.

Centesimal composition

The developed foods were evaluated for moisture, ash, fibre, protein and lipids, according to the methodology described by AOAC (2000). The method of 3.5 DNS (Miller, 1959) was used to determine the carbohydrate content, with prior acid hydrolysis.

Sensory analysis

The sensory evaluation of the developed foods and of similar products found in the market was carried out through acceptance testing and purchase intent. The panel of assessors was composed of 40 individuals, practitioners of physical activities, of both genders. After rehydration with milk, the products were presented in a random order to the panellists in plastic glasses (with a three digit code) at 4°C (Valero-Cases and Frutos, 2017).

The sensory panellists were not informed of which sample contained *Spirulina* sp. LEB 18. A hedonic scale of nine points was used to evaluate the acceptance of the products, in which point 1 corresponded to "extremely dislike" and point 9 corresponded to "extremely like". The purchase attitude of the panellists was verified using a five-point scale from "certainly would not buy" (point 1) to "certainly would buy" (point 5) (Meilgaard *et al*., 2015).

Estimated shelf life of the products

The shelf life was estimated according to the mathematical model that relates the moisture increase of the product through its moisture sorption isotherm with the water vapour transmission rate (WVTR) of the packaging (Alves *et al*., 1996). The model is described by Equation 1, where SL was the estimated shelf life (d); md was the dry mass of product (g); RH was the relative humidity of storage environment (%); WVTR was the water vapour transmission rate of the packaging ($g_{H2O}.m^{-2}.d^{-1}$); a_w (M) was the water activity of the product as a function of moisture content (moisture sorption isotherm of the product); M_0 and M_c were initial and critical moisture of the product (% $w.w^{-1}$) determined according to AOAC (2000), and A was packaging area (m^2).

$$SL = \frac{md.RH}{100.A.WVTR} \int_{Mo}^{Mc} \frac{dM}{\frac{RH}{100} - aw(M)} \quad (1)$$

Water vapour transmission rate (WVTR)

High-density polyethylene packaging - HDPE (Model R30, InjePlast) were characterised for the WVTR according to the methodology of ASTM E96/

E96-05 (2005). This method is based on increased mass of anhydrous calcium chloride ($CaCl_2$) stored in packaging. For this, they were placed in a desiccator with a saturated solution of $BaCl_2$ (RH = 90%) at 30°C. Weight gain of $CaCl_2$ was determined daily until constant. The WVTR ($gH_2O/m^2.d$) of packaging was calculated according to Equation 2, where G/t (gH_2O/d) was the angular coefficient of the linear adjustment of mass gain curve as a function of time (t), and A (m^2) was the permeation area of the package.

$$WVTR=\frac{(G/t)}{A} \tag{2}$$

Moisture sorption isotherm and critical humidity

To determine the moisture sorption isotherm, the samples were placed in desiccators containing saturated salt solutions (CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, NaCl and $BaCl_2$) with RH between 22 and 90%. The desiccators were maintained at 30°C until the mass of the samples stabilised.

After this period the equilibrium moisture content (M) was determined according to AOAC (2000) for each RH condition. Experimental sorption isotherm data were adjusted by Halsey equation (Equation 3), where C_1 and C_2 are adjustment constants (Halsey, 1948). The critical moisture of the product was based on the changes that have occurred during storage at 30°C, in different RH conditions.

$$a_w=\exp\left(-\frac{C_1}{M^{C_2}}\right) \tag{3}$$

Statistical analysis

The results of sensory testing and chemical composition were evaluated by Analysis of Variance with 95% confidence interval and mean difference by Tukey's test.

Results and discussion

Centesimal composition

A reduced carbohydrate content (8.6%) and increased lipid content (29.3%) was observed for the meal replacement shake with *Spirulina* sp. LEB 18 when compared to the product without microalga (Table 1). The increased concentration of lipids in the products enriched with *Spirulina* might be favourable, since the biomass of microalga is considered a source of essential fatty acids and/or polyunsaturated fatty acids (Simsek *et al*., 2007).

The protein content for both developed foods was approximately 20% ($w.w^{-1}$). Santos *et al*. (2016) added *Spirulina* in foods for the elderly and they observed that the *Spirulina*-enriched food can be considered a source of protein and carbohydrate, which contributes to the energy and nutrient requirements of elderly people. Rabelo *et al*. (2013) found that the addition of *Spirulina* platensis in products such as donuts promoted an increase in protein content. The formulation with an increase of 5.4% ($w.w^{-1}$) of S. platensis increased 66.7% protein content relative to the standard formulation. Morais *et al*. (2006) developed chocolate cookies enriched with S. platensis and observed that with the addition of 5.0% ($w.w^{-1}$) of biomass, protein content was 7.7% higher than the control. Thus, higher concentrations of microalgal biomass in the formulations of the products developed could allow an increase in protein content.

Spirulina biomass has been used in vitamin supplements and included in nutritional drinks because this microalga contains vitamins and minerals, and its use contributes to health, body regulation and favours of the immune system (Zeng and Liang, 1995; Madhyastha *et al*., 2006; Chu *et al*., 2010; Nuhu, 2013; Agustini *et al*., 2015). *Spirulina* sp. LEB 18 biomass usually presents larger amounts of essential amino acids like isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan

Table 1. Proximate Composition (%, $w.w^{-1}$) of the developed products

Components	High-calorie food supplement with *Spirulina*	High-calorie food supplement without *Spirulina*	Shake with *Spirulina*	Shake without *Spirulina*	*Spirulina* biomass (Lucas *et al*., 2017)
Ashes	2.91 ± 0.07^a	2.78 ± 0.01^a	3.54 ± 0.01^a	3.50 ± 0.04^a	16.50
Lipids	2.79 ± 0.03^b	2.68 ± 0.27^b	4.24 ± 0.05^b	3.28 ± 0.13^c	7.00
Proteins	18.17 ± 1.25^c	16.99 ± 0.43^c	19.47 ± 0.35^d	19.87 ± 0.69^d	59.50
Carbohydrates	69.47 ± 4.00^d	73.98 ± 0.11d	81.34 ± 0.74^e	88.98 ± 0.62^f	17.20
Fibres	0.65 ± 0.09^e	0.54 ± 0.13^e	0.37 ± 0.05^g	0.44 ± 0.08^g	-

-: Not available; Results presented on a dry basis. Same letters in the same line for each product, indicate that the averages do not differ significantly (p> 0.05) by Tukey test.

and valine, than other *Spirulina* strains (Morais, *et al*., 2009). Moreover, the *Spirulina* biomass contains vitamins as B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_6 (pyridoxine), B_{12} (Analogue), E, K, folic acid, inositol, pantothenate biotin, and presents minerals as calcium, potassium, magnesium, sodium, phosphorus, copper, iron, manganese, zinc, chromium and selenium (Morsy *et al*., 2014). Thus, the incorporation of *Spirulina* biomass in high-calorie food supplements, and meal replacement shakes becomes advantageous due to its nutritional characteristics.

Sensory analysis

The meal replacement shakes with and without *Spirulina* sp. LEB 18 had higher averages compared to the commercial product (Table 2), corresponding, on a hedonic scale between "like" and "like regularly." With regard to the evaluated high-calorie food, the product without the addition of *Spirulina* sp. LEB 18 received the highest average points from physical activity practitioners. This difference can be explained by the presence of characteristic colour and flavour of the microalgal biomass, little recognised by the target group. The average of the high-calorie food with the incorporation of *Spirulina* sp. LEB 18 showed no difference from the commercial product.

Table 2. Average points awarded by panellists to the developed products

Product	Average Points		
	Commercial	With *Spirulina*	Without *Spirulina*
High-calorie supplement	6.36 ± 2.07^b	6.86 ± 1.99^b	8.05 ± 1.03^a
Shake	6.12 ± 1.65^b	7.19 ± 1.56^a	7.60 ± 1.12^a

Same letters on the same row indicate that averages do not differ significantly ($p > 0.05$) by Tukey test.

Among the 40 panellists who evaluated the samples, over 65.0% had a purchase attitude between "definitely buy" and "probably buy" for both products developed with the addition of *Spirulina* sp. LEB 18 (Figure 1). Lemes *et al*. (2012) evaluated the enrichment of S. platensis in wheat flour for the development of pasta. The sensory quality of this product was considered satisfactory ("liked") and high purchase intent ("probably buy").

The commercial high-calorie food supplement had 27.3% and the commercial meal replacement shake presented 34.2% purchase attitude between "would certainly not buy" and "would probably not buy". Thus, the probable purchase intent of the formulations of foods incorporated with *Spirulina* sp. LEB 18 was evident. Moreover, these results are consistent with the acceptance test.

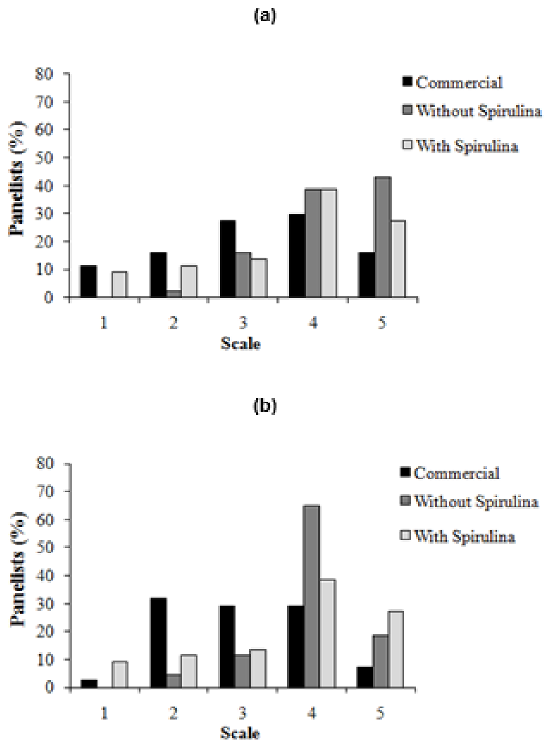

Figure 1. Purchase intent of the high-calorie food supplement (a) and meal replacement shakes (b) by the panellists (1 - would definitely not buy; 2 - would probably not buy; 3 - I might buy, 4 - would probably buy; 5 – would definitely buy).

Carvalho *et al*. (2016) developed similar products to this work. The authors developed six types of foods for practitioners of physical activity as an electrolyte replenisher, a muscle recovery supplement, and a muscle enhancer. These foods were produced with (0.5%, $w.w^{-1}$) and without the addition of *Spirulina*. The authors evaluated the acceptance and purchase intent of these foods front of the target group. With regard to purchase intent among the 40 panellists, 79.5%, 87.5% and 58.5% of the panellists had a positive attitude towards the purchase of products with *Spirulina* (electrolyte replenishers, muscle enhancers and muscle recovery, respectively). In addition, all developed products in the present work (meal replacement shake and a high-calorie food) with *Spirulina* sp. LEB 18 were accepted by the target public with acceptance above 70%.

Table 3. Constants (C_1 and C_2) and correlation coefficient (R) of Halsey Equation

Product	C_1	C_2	R
High-calorie supplement without *Spirulina*	3.94	0.77	0.99
High-calorie supplement with *Spirulina*	3.99	0.77	0.99
Shake without *Spirulina*	5.45	0.89	0.99
Shake with *Spirulina*	5.12	0.86	0.98

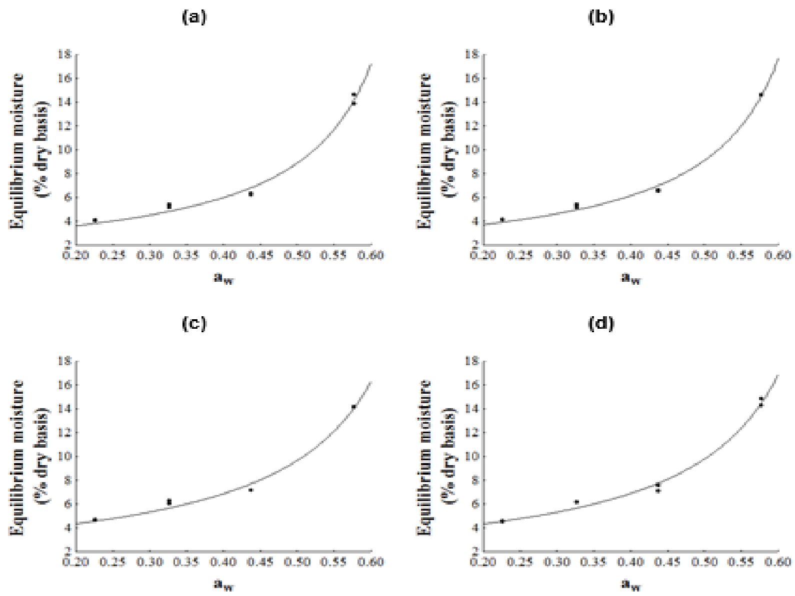

Figure 2. Sorption moisture isotherms of high-calorie food supplement (a) without and (b) with *Spirulina*, and meal replacement shake (c) without and (d) with *Spirulina*.

Shelf life estimation

The HDPE packaging presented a WVTR of 0.8 $gH_2O.m^{-2}.d^{-1}$. The correlation coefficients (Table 3) indicate appropriate settings for moisture sorption isotherms (Figure 2). With this, the shelf life of developed products was estimated by Equation 1, which related the increased moisture of the product by its sorption isotherm with the WVTR of the package.

Through the changes observed, such as darkening and agglomeration of the samples, the critical moisture of 0.43 was determined in water activity. For the high-calorie food supplement, the critical moisture was 6.34 and 6.11% ($w.w^{-1}$) for products with and without the addition of *Spirulina* sp. LEB 18, respectively. The critical moisture of the meal replacement shake added with microalgal biomass was 7.23% ($w.w^{-1}$) and without *Spirulina* sp. LEB 18 the value was 7.03% ($w.w^{-1}$).

Integrating the equation in the interval between the initial moisture of the high-calorie food with *Spirulina* sp. LEB 18 (5.13%, $w.w^{-1}$) and its critical moisture, the shelf life of this product was estimated at 26 months. The meal replacement shake with *Spirulina* sp. LEB 18 showed initial moisture of 6.49% ($w.w^{-1}$) and a shelf life of 17 months.

In foods without the addition of microalgal biomass, the initial moisture content was 4.92% ($w.w^{-1}$) for the high-calorie food and 6.23% ($w.w^{-1}$) for the meal replacement shake, with an estimated shelf life of these foods being 25 and 18 months, respectively. In both developed foods, the results of the shelf life for products with and without the addition of microalga were similar. Santos *et al*. (2016) also assessed the shelf life of foods with added *Spirulina* through a mathematical model and the estimated shelf life was equivalent to similar commercialised foods. Therefore, the biomass of *Spirulina* can be used in the formulations of these foods without damaging the shelf life of the product.

Other studies were performed with mathematical models to estimate the shelf life of foods (Mataragas *et al*., 2011; Escobedo-Avellaneda *et al*., 2012; Oliveira *et al*., 2012). Amodio *et al*. (2014) assessed the shelf life of fresh rocket leaf salad using the Weibullian model to fit the experimental data and obtained a correlation coefficient between 0.95 and 0.99. Thus, the shelf life of foods with *Spirulina* sp. LEB 18 (high-calorie food supplement and the meal replacement shake) can also be seen with the use of mathematical models.

Conclusion

The major constituents of the meal replacement shake and a high-calorie food supplement, both with *Spirulina* sp. LEB 18, were carbohydrates and proteins. The addition of microalgal biomass promoted an intake of carbohydrates without affecting the acceptance of the developed products. The shelf life of biomass-enriched foods was estimated at 26 months for the high-calorie food supplement and 17 months for meal replacement shake. Therefore, the incorporation of *Spirulina* sp. LEB 18 biomass in the products is able to meet the energy and nutritional needs of practitioners of physical activities.

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References

Agustini, T. W., Suzery, M., Sutrisnanto, D. and Ma'ruf, W. F. 2015. Comparative study of bioactive substances extracted from fresh and dried *Spirulina* sp. Procedia Environmental Sciences 23: 282-289.

Alves, R. M. V., Bordin, M. R. and Garcia, E. E. C. 1996. Application of a mathematical model in the estimation of shelf life of "cream cracker". Brazil: Collection of Food Technology Institute - ITAL.

Amodio, M. L., Derossi, A., Mastrandrea, L. and Colelli, G. 2014. A study of the estimated shelf life of fresh rocket using a non-linear model. Journal of Food Engineering 150: 19-28.

AOAC. 2000. Official methods of analysis of the Association of Official Analytical Chemists International. 17th ed. Gaithersburg: Association of Analytical Communities.

ASTM. 2005. Standard test methods for water vapor transmission of materials. Philadelphia: American Society for Testing and Materials.

Batista, A. P., Nunes, M. C., Fradinho, P., Gouveia, L., Sousa, I., Raymundo, A. and Franco, J. M. 2012. Novel foods with microalgal ingredients – Effect of gel setting conditions on the linear viscoelasticity of *Spirulina* and Haematococcus gels. Journal of Food Engineering 110(2): 182-189.

Becker, E. W., Jakober, B., Luft, D. and Schmulling, R. M. 1986. Clinical and biochemical evaluations of the alga *Spirulina* with regard to its application in the treatment of obesity. A double-blind cross-over study. Nutrition Reports International 33: 565-574.

Belay, A., Ota, Y., Miyakawa, K. and Shimamatsu, H. 1993. Current knowledge on potential health benefits of *Spirulina*. Journal of Applied Phycology 5(2): 235-241.

Bird, S. P., Tarpenning, K. M., Marino and F. E. 2006. Effects of liquid carbohydrate/essential amino acid ingestion on acute hormonal response during a single bout of resistance exercise in untrained men. Nutrition 22 (4): 367–75.

Carvalho, L. F., Moreira, J. B., Oliveira, M. S. and Costa, J. A. V. 2016. Acceptance of novel foods for physical activity practitioners formulated with *Spirulina*. IOSR Journal of Biotechnology and Biochemistry 2(4): 7–11.

Chu, W. L., Lim, Y. W., Radhakrishnan, A. K. and Lim, P. E. 2010. Protective effect of aqueous extract from *Spirulina* platensis against cell death induced by free radicals. BMC Complementary and Alternative Medicine 10:53.

El-Tantawy, W. H. 2015. Antioxidant effects of *Spirulina* supplement against lead acetate-induced hepatic injury in rats. Journal of Traditional and Complementary Medicine 6(4): 327-331.

Escobedo-Avellaneda, Z., Velazquez, G., Torres, J. A. and Welti-Chanes, J. 2012. Inclusion of the variability of model parameters on shelf-life estimations for low and intermediate moisture vegetables. LWT - Food Science and Technology 47(2): 364–370.

Food and Drug Administration (FDA). 2012. Center for Food Safety and Applied Nutrition. Office of Food Additive Safety. Agency Response Letter - GRAS Notice No. 000417. Retrieved on August 31, 2017 from FDA Website: https://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm319628.htm

Galli, N. and Reel, J. J. 2009. Adonis or Hephaestus? Exploring body image in male athletes. Psychology of Men and Masculinity 10(2): 95-108.

Halsey, G. 1948. Physical adsorption on non-uniform surfaces. The Journal of Chemical Physics 16(10): 931-937.

Hosoyamada, Y., Takai, Y. and Kato, T. 1991. Effects of water-soluble and -insoluble fractions of *Spirulina* on serum lipid components and glucose tolerance in rats. Journal of Japan Society of Nutrition and Food Sciences 44: 273-277.

Lemes, A. C., Takeuchi, K. P., de Carvalho, J. C. M. and Danesi, E. D. G. 2012. Fresh pasta production enriched with *Spirulina* platensis biomass. Brazilian Archives of Biology and Technology 55(5): 741–750.

Li, D and Qi, Y. 1997. *Spirulina* industry in China : Present status and future prospects. Journal of Applied Phycology 9: 25-28.

Lu, H. K., Hsieh, C. C., Hsu, J. J., Yang, Y. K. and Chou, H. N. 2006. Preventive effects of *Spirulina* platensis on skeletal muscle damage under exercise-induced oxidative stress. European Journal of Applied Physiology 98(2): 220-226.

Lucas, B. F., Morais, M. G., Santos, T. D. and Costa, J. A. V. 2017. Effect of *Spirulina* addition on the physicochemical and structural properties of extruded snacks. Food Science and Technology 37(suppl): 16-23.

Madhyastha, H. K., Radha, K. S., Sugiki, M., Omura, S. and Maruyama, M. 2006. Purification of c-phycocyanin from *Spirulina* fusiformis and its effect on the induction of urokinase-type plasminogen activator from calf pulmonary endothelial cells. Phytomedicine 13(8): 564-569.

Mataragas, M., Dimitriou, V., Skandamis, P. N. and Drosinos, E. H. 2011. Quantifying the spoilage and shelf-life of yoghurt with fruits. Food Microbiology 28(3): 611-616.

Meilgaard, M., Civille, G. V. and Carr, B. T. 2015. Sensory Evaluation Techniques. 5th ed. Florida: CRC Press.

Miller, G. L. 1959. Use of dinitrosalicylic acid for determination of reducing sugar. Analytical Chemistry 31(3): 420-428.

Morais, M. G., Miranda, M. Z. and Costa, J. A. V. 2006. Chocolate cookies enriched with *Spirulina* platensis: physical-chemical, sensory and digestibility characteristics. Alimentos e Nutrição Araraquara 17(3): 323-328.

Morais, M. G., Radmann, E. M., Andrade, M. R., Teixeira, G. G., Brusch, L. R. F. and Costa, J. A. V. 2009. Pilot scale semicontinuous production of *Spirulina* biomass in southern Brazil. Aquaculture 294(1-2):60-64.

Morsy, O. M., Sharoba, A. M., EL-Desouky, A. I., Bahlol, H. E. M. and Abd El, M. E. M. 2014. Production and evaluation of some extruded food products using *Spirulina* algae. Annals of Agricultural Science 52(4): 329-342.

Nuhu, A. A. 2013. *Spirulina* (*Arthrospira*): An important source of nutritional and medicinal compounds. Journal of Marine Biology 2013: 1-8.

Oliveira, F., Sousa-Gallagher, M. J., Mahajan, P. V. and Teixeira, J. A. 2012. Development of shelf-life kinetic model for modified atmosphere packaging of fresh sliced mushrooms. Journal of Food Engineering 111(2): 466-473.

Pope, Z., Gao, Y., Bolter, N. and Pritchard, M. 2015. Validity and reliability of eating disorder assessments used with athletes: A review. Journal of Sport and Health Science 4(3): 211-221.

Rabelo, S. F., Lemes, A. C., Takeuchi, K. P., Frata, M. T., Carvalho, J. C. M. and Danesi, E. D. G. 2013. Development of cassava doughnuts enriched with *Spirulina* platensis biomass. Brazilian Journal of Food Technology 16(1): 42-51.

Ramamoorthy, A. and Premakumari, S. 1996. Effect of supplementation of *Spirulina* on hypercholesterolemic patients. Journal of Food Science and Technology 33(2):124–128.

Santos, T. D., Freitas, B. C.B., Moreira, J. B., Zanfonato, K. and Costa J. A. V. 2016. Development of powdered food with the addition of *Spirulina* for food supplementation of the elderly population. Innovative Food Science and Emerging Technology 37:216–220.

Simsek, N., Karadeniz, A. and Karaca, T. 2007. Effects of the *Spirulina* platensis and Panax ginseng oral supplementation on peripheral blood cells in rats. Revue de Médecine Vétérinaire 158(10): 483-488.

Tipton, K. D., Rasmussen, B. B., Miller, S. L., Wolf, S. E., Owens-Stovall, S. K., Petrini, B. E. and Wolfe, R. R. 2001. Timing of amino acid-carbohydrate ingestion alters anabolic response of muscle to resistance exercise. American Journal of Physiology-Endocrinology and Metabolism 281(2): 197–206.

Valero-Cases, E. and Frutos, M. J. 2017. Development of prebiotic nectars and juices as potential substrates for Lactobacillus acidophilus: Special reference to physicochemical characterization and consumer acceptability during storage. LWT - Food Science and Technology 81: 136-143.

Walker, T. L., Purton, S., Becker, D. K. and Collet, C. 2005. Microalgae as bioreactors. Plant Cell Reports 24(11): 629-641.

Zeng, Y. and Liang, M. S. 1995. Production of *Spirulina* drink. Food Science 16(7): 39-41.