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Research Article

Algae protein enriched nutritious snacks and their sensory evaluation

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Abstract

Protein is an important component of human nutrition. Algal protein is a novel protein supplement, which has amino acid composition like that of soy protein which is a commonly used plant protein source. In this paper, purified protein from a green alga, Picochlorum sp., is incorporated in popular snacks such as, tortilla chips, energy bar and khakhara, as a protein supplement. The algal protein used in the snacks is 80-85% pure containing ~32% of essential amino acids. The algal protein is added at 5% concentration and improves the total protein in the tortilla chips, energy bar and khakhara, by 44%, 40% and 34%, respectively. The carbohydrate content is reduced in the algae protein integrated tortilla chips, khakhara and energy bar by 4.6%, 6.4% and 6.9%, respectively. The sensory evaluation of the test and control samples against the essential attributes for the three snack items showed a good acceptability of the algal protein- incorporated tortilla chips, khakhara and energy bar. Hence, this will be helpful in catering to protein deficiency problems and adds to the options for consumers interested in vegan protein sources.

Keywords: Algae Protein; Tortilla Chips; Khakhara; Energy Bar; Novel food; Snacks; Sustainable food

1. Introduction

Food is an integral part of human culture and has continuously evolved with growing needs of nutrition [1]. Protein in the daily diet is not only the basic building block for the body tissues but also boosts immunity and helps the body to fight diseases (https://www.pcrm.org/news/blog/foods-boost-immune-system). With the millennials becoming more careful and vigilant about the nutritional status of their food, there are novel and healthier versions of foodstuffs continuously available in the market [2]. The newer generation is more open to healthier food options especially protein which is an important nutrient for human body. In addition, protein deficiency is a growing concern in several countries of the world [3]. Till now plant protein especially soy protein was the most common protein source for vegetarians used in various food products [4]. However, non-vegetarians could fulfill their protein needs by various animal protein sources. These protein sources contain essential and non-essential amino acids important for growth, and as a source of structural protein and enzymes [4]. Global population growth is stretching the limit of food production capacity and adversely affecting the environment. Livestock derived products is cause of concern due to lack in sustainability. Hence, need of sources of alternative proteins and other food ingredient [5]. For thousands of years, microalgae have been used as dietary supplements without any significant side effects [6,7]. Recently, algae are being used for production of nutraceuticals apart from other high value products [8-10]. Algal proteins have an amino acid composition similar to soy protein, one of the most common plant protein sources [11,12]. The amount of essential amino acids in algae are also comparable to that of soya proteins [11,12]. *Spirulina* and *Chlorella* proteins are used as protein supplements and functional foods owing to their vitamin and mineral enriched protein having numerous therapeutic properties [13]. *Spirulina* protein is approximately 60% of the total biomass [14] and has anticancer, antiviral, antioxidant and anti-inflammatory properties [15]. It also has vitamins C, B6 and B12 and high amount of dietary fiber (https://www.antenna.ch/wp-content/uploads/2017/03/AspectNut_UK.pdf). It has been stated by Sanaraj and Sivaskati [16] that 'one gram of *Spirulina* protein is equivalent to one kilogram of assorted vegetables. *Chlorella* contains approximately 55-60% protein, which has omega-3 fatty acids, vitamins (B12), minerals and iron. *Chlorella* protein has numerous therapeutic properties such as antioxidant, antidiabetic and helps in improving immune response. Recently, innovative foods have been in vogue. The consumers want to embrace the most innovative healthy food options [17,18]. Many sea weeds/vegetables such as Porphyra, Palmaria and Alaria are being used in European cuisines, which not only impart distinct flavor and texture but also add protein, vitamins and minerals to improve the nutritive value of the food [19]. In addition, algal protein is the most suitable option for consumers who want to switch to vegan diets. This has led to the utilization of algae in various food products such as energy bar, cookies, pasta, sushi, probiotics, bread, yoghurts, etc [10,20]. Supplementation with algal protein not only enhances the protein content but also forms a wholesome nutritive snack/food product. Tortilla chips is an important flavorful snack prepared using corn flour. Khakhara is an Indian snack which is savoured for its rich taste all over the world. On the other hand, energy bar is suited for high energy snacking as an 'on the go' food item. Typically, snacks are dense calorie foods consisting of high carbohydrate and fat content, but with respect to the amount of protein content, they have low nutritional value. Thus, most snacks are referred to as "empty calorie" foods as they provide less than 2% protein nutrition [21]. Cereals are the biggest source of protein and energy in human diet. Supplementing cereals with protein in the tortilla chips, khakhara and energy bars, increases its nutritive

value making it a complete snack having carbohydrate, fat, and protein. In earlier studies, many other different supplements such as broccoli, finger millets and other nutrient sources, have been tested to make tortilla chips [22], khakhara [23,24] and energy bar [25] more nutritious snacks. In this paper, we demonstrate the culinary and cooking characteristics by an integration of 5% algal protein extracted at the production facility of Reliance Industries Limited, for the preparation of tortilla chips, khakhara and energy bar. The algal protein integrated tortilla chips, khakhara and energy bar are evaluated using sensory analysis against the control tortilla chips, khakhara and energy bar, respectively.

2. Materials and Methods

2.1 Strain and growth conditions

Microalgae was grown phototrophically as described by Karuppasamy et al. [26] and Rajvanshi et al. [27]. The protein from algae was extracted and specifications of the protein was supplied by the Algae production unit of the company. Algal protein was analyzed for protein purity ultimate analysis, ash content and lipid content.

2.2 Tortilla chips

2.2.1 Preparation of Dough for Control Tortilla Chips Sample: For the preparation of control samples, 66 g of corn flour was mixed with 33 g of wheat flour. Turmeric powder (250 mg) was added to impart yellow colour and 750 mg of salt was added to it. The mixture was combined by adding approximately 200 mL of lukewarm water. The dough was kneaded for 15 minutes. 5 mL of oil was used to coat the dough, which was allowed to rest for 30 minutes after covering with cling foil.

2.2.2 Preparation of dough for algal protein integrated Tortilla Chips (Test Sample): In the test sample, all ingredients are same as in the control sample except that 5 g of algae protein and 28 g of wheat flour were added.

2.2.3 Preparation of Tortilla Chips: The recipe for the preparation of Tortilla chips was used from [28] with some modifications. The dough was divided into five equal sized balls. Each ball was rolled out to form a circular sheet of 14-15 cm to a thickness of 1.5 mm. The triangular shape of the tortilla chips was cut such that each side was 4 cm. A fork was used to pierce the entire sheet to prevent the tortilla chips from swelling on frying. 300 mL oil was taken in a pan, which was heated to a temperature of 180 ºC. The tortilla triangles were fried on medium hot oil for 12 minutes. The fried chips were then taken out from oil and drained on paper towels to get rid of extra oil. A spice mix of garlic powder, salt, paprika in equal ratio was sprinkled on the chips immediately to ensure better sticking to the chips.

2.3 Energy bar

2.3.1 Preparation of control sample: Manually, 15 g dates peeled to remove the seeds. Pulp was soaked into water in the ratio of 1: 2 on weight basis (pulp: water) for one hour. Excess water removed with help of tissue paper. This pulp was grinded into smooth paste like consistence. Followed with low heat cooking of pulp paste to get the syrup like consistency. To this syrup added rolled oats (25 g), almond (10 g), cashew (10 g), honey (5 g), raisins (5 g), ghee (2 g), natural gums (5 g), cocoa powder (2 g) and vegetable oil (5 g). This mixture transferred on an oiled tray and leveled with a roller pin. After cooling, rolled mixture pieces into rectangular bars of approximately 25 g each.

2.3.2 Preparation of test sample: In the test sample, all ingredients were same as in the control sample except that 5 g of white algae protein was replaced with 5 g rolled oats.

2.4 Khakhara

2.4.1 Preparation of Dough for Khakhara (Control Sample): For the preparation of control samples, 61 g of whole wheat flour was mixed with 1 g whole sesame seeds, 1 g salt, 1 g chat masala. To it 10 g sunflower oil was added. The mixture was combined by adding 18g of water. The dough was kneaded for 5 minutes. 2 mL of oil was used to coat the dough, which was allowed to rest for 30 minutes after covering with lid.

2.4.2 Preparation of dough for algal protein Khakhara (Test Sample): In the test sample, all ingredients are same as in the control sample except that 5 g of algae protein is replaced with wheat flour.

2.4.3 Preparation of Khakhara: The recipe for the preparation of khakhara was used from [29] with some modifications. The dough was divided into five equal sized balls. Each ball was rolled out to form a circular sheet of 15 cm to a thickness of 2.0 mm. The khakhara was roasted on medium flame using flat tawa until golden brown.

2.5 Nutrition calculations

The recipe information for Tortilla chips, Energy Bar and Khakhara were were fed in an online software to calculate the nutritional panel for the test and control samples. The nutrition labels were calculated using online 'VeryWellfit' Recipe nutrition calculator (https://www.verywellfit.com/recipe-nutrition-analyzer-4157076). The values obtained for various nutrients was used to calculate the percentage increase or decrease in the nutrients in the test samples.

2.6 Ultimate analysis/CHNSO and amino acid analysis

The CHNSO and amino acid analysis was performed on three replicates for the treated algal protein sample. The ultimate analysis of the protein powder was carried out by CHNSO analyser (Vario MACRO cube, Elementar GmBH, Germany). The amino acid analysis of the protein powder was performed by Acquity H-class (Model- A17QSM590A) UPLC instrument fitted with PDA detector (Waters Corporation). The hydrolysis of the sample was performed with 6N HCl in a vacuum oven (Thermo scientific) at 110°C prior to derivatization.

2.7 Sensory evaluation

The samples were prepared almost 12 hours before the sensory evaluation was performed. The control and test samples were stored in airtight containers to keep the crunchiness and flavor intact. The samples were evaluated on a 9-point hedonic scale by a panel of 10 untrained ordinary consumers [30]. The ordinary consumers were selected from the age group of 30-60 years. The tasting tests were performed in a special room and the test and control samples were kept at room temperature in air-tight boxes. All the three snacks were tested at different time to avoid cross-contamination between samples. The responses were made against comparison with the control tortilla chips on a scale of 1 to 9 where 1 refers to Dislike Extremely followed by Dislike Very Much (2), Dislike Moderately (3), Dislike Slightly (4), Neither Like nor Dislike (5), Like Slightly (6), Like Moderately (7), Like Very Much (8) and Like Extremely (9). The results of the evaluation were summarized in a spider web chart.

3. Results

3.1 Algal protein analysis

CHNS analysis shows that the nitrogen percent of the algal protein powder was 13.4% (Table 1). The total protein content of the powder was found to be 80-85% that was calculated using a conversion factor for algae protein identified through earlier studies. The protein powder was devoid of any moisture and contained minimal amounts of carbohydrate, lipid, and ash.

Constitution	Amount (%)
N%	13.4 ± 0.89
C%	47.7 ± 1.58
H%	6.5 ± 0.13
S%	1.6 ± 0.12
Total Amino Acid Content	80-85
Essential Amino Acid Content	~32

Table 1. Ultimate analysis of algal protein powder and its amino acid content.

3.2 Preparation of algal protein integrated Tortilla chips, Energy Bar and Khakhara

The test samples were prepared using 5% of algal protein with specifications supplied by the Algae production unit of RIL. The integration of algal protein to the tortilla and khakhara dough imparted a green colour (Supplementary figure 1), which could also be seen in the final product after frying (Figure 1). The algal protein powder was lighter in colour, however, on mixing with water the green colour became deeper. Further, the refined oil used for frying the tortilla test samples turned slightly green. This could be because the protein bound to pigments such as chlorophyll, which also got extracted. The pigments are polar lipids, which bound to the frying oil at higher temperature and leached out of the chips. The control sample (100 g) contains 8 g of protein whereas addition of 5% algal protein increases the protein content to 11.5 g. Hence, using this algal protein, the protein content was enhanced by 44% in the tortilla test samples as compared to the control samples (Table 2). The nutritional panel also showed the reduction in the carbohydrate content by 4.6% in the algae protein integrated tortilla chips samples. Addition of algal protein also improved the iron content by 33% as compared to the control samples. There was some greenish hue and grassy smell imparted to the tortilla chips due to the addition of algal protein (Figure 2). In case of energy bar, the protein content was enhanced by 40% due to the addition of green protein and the carbohydrate content was increased by 6.4% in the test samples as compared to control energy bar samples (Table 2). Also, in the Indian snack, khakhara, the protein content was improved by 34% and the carbohydrate content reduced by 6.9% in the test sample as compared to control khakhara samples (Table 2). The iron content in the khakhara test and control samples reduced by 25%, however, the iron content in the energy bar test and control samples remained the same.

Figure 1: Image of Control and Algae protein fortified Tortilla Chips, Khakhara and Energy Bar.

Supplementary Figure 1: Khakhara dough showing the change in color of the dough after incorporation of algae protein.

Nutrition Facts (Serving size-100 g)						
	%Daily Value/ Amount per serving					
Snacks	Tortilla Chips		Energy Bar		Khakhara	
	Control	Test	Control	Test	Control	Test
Calorific Value (Calories)	359	358	325	323	326	327
Total Fat (%/g)	4/2.9	4/3	20/15.6	20/15.4	11/ 8.2	11/ 8.4
Saturated Fat (%/g)	2/0.4	2/0.4	16/3.2	16/ 3.1	4/0.9	4/ 0.9
Cholesterol (%/mg)	0	0	2/ 5	2/ 5	0	0
Sodium (%/mg)	13/295	15/345	6/140	8/190	17/392	17/392
Total carbohydrates (%/g)	28/76.1	26/72.6	17/46.8	16/43.8	20/54.4	18/ 50.6
Dietary fiber (%/g)	21/5.8	21/5.9	36/10.1	35/9.9	7/2	7/ 1.9
Protein (%/g)	13.3/8	19.1/11.5	13.6/8.2	19.1/11.5	12.4/7.5	18.9/11.4
Calcium (%/g)	1/10	1/18	5/59	5/66	2/21	2/20
Iron (%/mg)	18/3	21/4	15/3	18/3	20/4	18/3
Potassium (%/mg)	5/250	5/248	9/409	8/395	2/81	2/76

Table 2: Nutrition facts of tortilla chips, energy bar and khakhara with and without Algae protein fortification generated using VeryWellfit nutrition calculator. The daily value of protein is calculated using the protein daily intake of an average human (60 g).

3.3 Sensory Evaluation

For sensory studies, the essential properties of each food product were identified and assigned a weightage based on their importance in the three snack items. Table 3 gives the essential properties/ attributes and their weightage for which the three snack items were evaluated. The sensory analysis for the prototypes was done and the results were measured across 9-point hedonic scale with '1' being least acceptable to '9' being most acceptable. The results of the sensory evaluation are summarized in the form of the spider web chart (Figure 2). The analysis clearly shows that with 5% integration of algal protein sample, the test samples were as good as the control samples in terms of flavor, mouthfeel, crunchiness. The test samples for tortilla and khakhara scored less in smell and flavor due to the presence of green colour imparted by lipids and pigments. However, based on the sensory analysis, the tortilla chips test sample was considered more nutritional as the nutrition status was greatly enhanced with 44% improvement in the protein content. Thus, the overall rating for the control and the test samples was same i.e. 'Like very much' on the 9-point hedonic scale (Figure 2). In case of Energy bar, appearance of control (without microalgae protein) and sample (with microalgae protein) were received same score from the panelist. Flavor, mouthfeel and texture profile of the control and test samples were same with each other. This indicates that addition of microalgae proteins did not alter the appearance, flavor, and texture properties of energy bar. Odour and crunchiness score was higher for control energy bar sample compared to test sample. Color of the test sample was found more acceptable as compared to the control energy bar sample. This might be due to microalga protein that helped in developing the color to final product energy bar which was relatively more acceptable. Nutritional value of test sample was higher compared to control because of additional microalgae proteins. Overall acceptability score of samples were same for control and test samples (Figure 2). The results of the sensory evaluation of khakhara control and test samples are summarized in the

form of the spider web chart (Figure 2). It was found that the test samples were as good as the control samples with respect to texture, mouthfeel, crunchiness. However, based on the sensory analysis, the khakhara test sample was considered more nutritional as the nutrition status was greatly enhanced with 34% improvement in the protein content. Thus, the overall rating for the control and the test samples was same i.e. 'Like very much' on the 9-point hedonic scale.

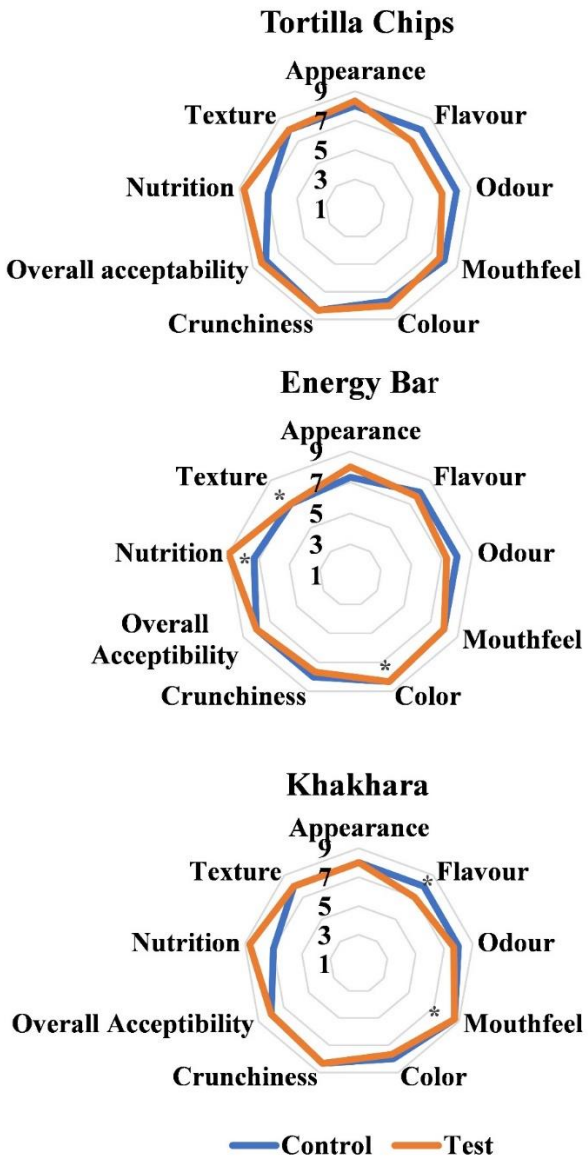

Figure 2: Spider diagram of descriptive sensory analysis of control and test samples of Tortilla Chips, Energy bar and Khakhara against nine sensory attributes. Statistical analysis was performed using ANOVA indicating significant differences between the control and test samples. The attributes showing significant difference between the test and control samples are indicated with an asterisk '*'.

Sensory Attributes	Tortilla Chips	Energy Bar	Khakhara
Appearance	++	++	++
Flavour	+++	+++	+++
Aroma/Odour	++	++	++
Mouthfeel	++	++	++
Colour	+	-	+
Crunchiness	+++	+	+++
Texture	++	++	++
Nutrition	+++	+++	+++
Softness	-	++	-
Aftertaste	++	++	++
Overall Acceptability	+++	+++	+++
'+' Important, '++' Very Important, '+++' Extremely Important, '-' Not important			

Table 3: List of attributes and their importance in sensory evaluation for each prototype. The attributes for each snack were identified from online sources and discussion with the sensory evaluation panel members.

4. Discussion

The ability of microalgae to fix CO_2 into biomass has attracted lot of attention in global market. Microalgae are rich source of protein containing amino acid, minerals, unsaturated fatty acids, beta carotene and multivitamins. The increasing number of people adopting health-conscious diet, vegan lifestyle, constant growth in population, increasing urbanization and economic developments worldwide are some of the major drivers to propel the algae protein market growth. EAAI of algal protein is 1.17 which is higher than soya protein. Higher EAAI indicates better amino acid profile for human consumption. From this, we can say that algal protein has comparatively better amino acid profile to that of soya protein [31,32]. However, further evaluation of the protein digestibility and benefits of algal protein on humans should be performed to arrive at conclusions. Algal protein has relatively better properties that allow it to integrate well with the ingredients of the snacks. However, chlorophyll/pigment bound proteins imparted yellow color to the dough of tortilla chips and khakhara. The effect of color of algal protein could not be seen in the energy bar being dark in colour. Also, at the time of frying, green colour of the protein was leached out of the chips, however, this could not be noticed in case of khakhara as it was dry roasted. Sensory evaluation of the test and control samples could clearly differentiate between the colour, texture and flavours in the test samples. The identification of sensory attributes for each snack aided in scoring of the test and control snacks. The snacks scored less in flavour which was imparted by the chlorophyll pigment bound proteins which imparted some off flavour. However, the colour of the test tortilla chips and khakhara was perceived to be more appetizing and hence, was more preferred.

5. Conclusion

The need of the consumers to switch to innovative, healthy, and vegan foodstuffs has led to the introduction of algae-based food products in the food market. The tortilla chips, khakhara and energy bars prepared in the above study demonstrated the optimal integration of algal protein at the concentration of 5% (dwb) with their other respective ingredients. Supplementation with algae protein improved the nutrition status of tortilla chips, energy bar and khakhara and rendered it a complete snack with 44%,

40% and 34% more protein. The sensory evaluation of the three snack items showed that these are under highly acceptable category using the nine-point hedonic scale. However, the test tortilla chips, khakhara and energy bars could be made more flavorful with less odor through the complete removal of pigments from the protein powder. This will involve additional steps of pigment extraction to make a colorless protein powder.

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