

# The Potential of *Porphyridium cruentum* in the Development of Gluten-Free Pancakes

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**Abstract**— Microalgae are natural and valuable products to cover food needs. *Porphyridium cruentum* is a red marine microalgae, capable of accumulating bioactive substances such as exopolysaccharides (EPS), polyunsaturated fatty acids (PUFAs), and R-phycoerythrin (R-PE). Gluten-free pancakes were made with 2% and 4% biomass of *P. cruentum*. Cooking loss, pH, moisture, ash, protein, texture profile analysis, and color were evaluated; a sensory evaluation was also conducted to assess color, smell, flavor, hardness, and overall acceptability. The sensory evaluation did not have significant statistical differences. There was also no difference in cooking loss or moisture content (%), but ash content (%) increased with increasing microalgae content. In the texture of the samples, there was no difference in cohesiveness. The use of this microalgae as a food ingredient is a promising form of enrichment and a feasible alternative for the addition in various products.

**Keywords**—Microalgae, sensory, color, gluten-free.

## I. INTRODUCTION

Celiac disease is a chronic autoimmune disorder, the symptoms of which are the result of ingestion of the major protein in wheat. This causes a chronic inflammatory process in the small intestine. As a possible solution to this condition, methods for obtaining proteins for food purposes from microorganisms have been sought [1].

In some countries, several species of microalgae are cultivated commercially, and the biomass obtained has been used for food applications. The market for functional foods, where microalgae are used in pastas, breads, yoghurts, and beverages, has developed rapidly in countries such as France, the United States, China, and Thailand [2]. Microalgae biomass is a natural source of biologically active compounds such as carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, and sterols [3]. Interest in health and safety issues related to the consumption of processed foods has increased; therefore, microalgae have become a trend as natural additives [4]. By incorporating them, health benefits can be obtained thanks to their bioactive compounds [5].

*Porphyridium cruentum* is a marine red microalgae that can synthesize bioactive compounds [6]. It has been studied for its ability to produce phycobiliproteins (PBPs) and PUFAs [7]. The FDA in the United States recognized *P. cruentum* as GRAS (Generally recognized as safe) [8].

### A. Justification

To add value to a traditional food by incorporating *Porphyridium cruentum* biomass, gluten-free pancakes were made. Therefore, it is of interest to analyze the application of microalgae in food.

### B. Objective

The present review aimed to evaluate the addition of the microalgae *Porphyridium cruentum* in gluten-free pancakes and to analyze consumer acceptance.

## II. MATERIALS AND METHODS

### A. Materials

Gluten-free flour was purchased in a shopping center, while the dry *Porphyridium cruentum* biomass was obtained from a culture of the Environmental Remediation Laboratory of the Agronomy Faculty of the Autonomous University of Nuevo León, which was maintained with continuous aeration. 26±2°C, photoperiod of 12 h, light intensity of 180 μmol m<sup>-2</sup> s<sup>-1</sup> and modified F2 [9] was used as culture medium.

### B. Preparation of pancakes

Three treatments were studied: control (commercial flour only), 2% (mixture containing 2% dry biomass of *P. cruentum*), and 4% (mixture containing 4% dry biomass of *P. cruentum*). The pancake formulation is shown in Table I.

TABLE I  
FORMULATION OF PANCAKES

Ingredients (g)	Control	2%	4%
Potato starch	85.93	84.22	82.50
Brown rice flour	84.15	82.47	80.78
Rice flour	63.48	62.23	60.96
Ground flaxseed	10.45	10.25	10.04
Chia	5.35	5.25	5.14

Baking powder	2.29	2.25	2.20
Xanthan gum	2.55	2.52	2.50
Grain salt	2.04	2.00	1.96
Stevia	3.00	3.00	3.00
Egg	126.00	126.00	126.00
Milk (mL)	362.00	362.00	362.00
<i>P. cruentum</i> biomass	0.00	5.10	10.2

The procedure for preparing the pancakes was to mix all the ingredients, weighing 50 g of dough for each pancake with a diameter of 8 cm. Subsequently, they were placed in a frying pan at 75+5°C, and cooking lasted 17 min on each side. Pancakes were cooled at ambient temperature for 20 min prior to further testing.

### C. Moisture

This method was based on method 930.15 [10] and consisted of evaporating the water from a sample under normal conditions.

### D. Ash

This method was based on method 942.05 [10] and consists of subjecting the sample to combustion to oxidize the organic matter; the resulting ashes are considered the mineral fraction [11].

### E. Cooking loss and pH

The pH measurement was performed according to method 943.02 [10]. To calculate the cooking loss (%) [12], the difference between the dough's post-cooking weight and the weight obtained was used.

### F. Protein

The protein content of the pancakes was determined by the Kjeldahl method based on method 976.05 [10].

### G. Color

The central part was measured with a colorimeter. Samples were measured on days 0 and 3. Analysis was based on the L\* a\* b\* system [13].

### H. Texture profile analysis

The texture of the samples was determined using a texture profile analysis (TPA) test with a Stable Micro Systems TA-XT Plus texturometer (Surrey, UK). In the analysis, the characteristics of hardness, cohesiveness, and chewiness were determined. Five repetitions of each treatment were performed on days 0 and 3 [14].

### I. Sensory evaluation

A 7-point hedonic test was conducted with 25 semi-trained panelists, where 1 corresponds to 'I dislike very much' and 7 to 'I like it very much'. The objective was to determine the degree of acceptance for smell, color, taste, hardness, and overall acceptability across the three

treatments. The samples were offered to the panelists 30 minutes after cooking [15].

## III. STATISTICAL ANALYSIS

The results were analyzed using Minitab 19, and a one-factor analysis of variance and Tukey's comparison of means were performed.

## IV. RESULTS AND DISCUSSION

### A. Moisture and ashes

Table II shows the results for moisture and ashes. The moisture content showed no statistical difference, whereas the ash content increased with increasing microalgae biomass. When gluten-free bread was made with *Spirulina platensis*, 1.92, 2.16, and 2.11% ashes were obtained for the control treatment, 2 and 4%, while for moisture it was 52.38, 51.67, and 49.06%, respectively [2]. This difference may be due to the variation in the type of sample.

TABLE II  
RESULTS OF MOISTURE AND ASHES

Treatments	Moisture (%)	Ashes (%)
Control	46.92±0.44 <sup>a</sup>	0.90±0.05 <sup>c</sup>
2%	47.08±0.79 <sup>a</sup>	1.29±0.05 <sup>b</sup>
4%	44.29±2.74 <sup>a</sup>	1.83±0.05 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

### B. Cooking losses and pH

The results are shown in Table III. There was no statistically significant difference in cooking loss. However, the pH of the samples increased with increasing biomass addition. One factor to consider is that the pH of the medium in which the microalgae grew was 8, and this can gradually increase as the cells grow due to CO<sub>2</sub> accumulation, which increases photosynthetic rate [16]. In addition, the cooking loss was higher than that reported in other research, with gluten-free bread containing *Chlorella sorokiniana* showing 13.4% in their treatments [17].

TABLE III  
RESULTS OF COOKING LOSS AND pH

Treatments	Cooking loss (%)	pH
Control	22.00±2.11 <sup>a</sup>	6.80±0.01 <sup>c</sup>
2%	22.20±3.05 <sup>a</sup>	8.02±0.04 <sup>b</sup>
4%	22.60±2.99 <sup>a</sup>	8.52±0.04 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

### C. Protein

This parameter was affected by the microalgae enrichment; all treatments differed significantly, as shown in Table IV. Although the control and 2% treatment decreased their protein content with cooking, adding 4%

microalgae biomass resulted in the highest protein content. Regarding protein content, microalgae breads increased their protein content; 3% w/w for *Nannochloropsis gaditana* and 18% for *Chlamydomonas* sp. [13]. Other research obtained 18% protein content in a gluten-free bread samples with 3% *Arthrospira platensis* biomass [2].

TABLE IV  
RESULTS OF PROTEIN

Treatments	% Protein	
	Uncooked	Cooked
Control	10.97±0.05 <sup>bc</sup>	10.65±0.10 <sup>c</sup>
2%	11.14±0.10 <sup>ab</sup>	10.88±0.10 <sup>bc</sup>
4%	11.13±0.04 <sup>ab</sup>	11.43±0.10 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

D. Color

Table V shows the color results for gluten-free pancakes. The luminosity was only statistically different in the 4% treatment at time 0. The control and 2% treatment decreased luminosity over the days of storage, whereas the 4% treatment increased it. When gluten-free bread was tested for color, lightness decreased upon the addition of brown algae [15].

TABLE V  
RESULTS OF COLOR

Treatments	L*	C*	H*
Day 0			
Control	45.66±3.21 <sup>a</sup>	29.14±2.62 <sup>a</sup>	63.06±2.84 <sup>a</sup>
2%	43.80±1.37 <sup>a</sup>	26.68±0.74 <sup>a</sup>	65.21±1.28 <sup>a</sup>
4%	38.31±0.87 <sup>b</sup>	22.19±0.51 <sup>b</sup>	62.39±2.26 <sup>a</sup>
Day 3			
Control	41.27±3.15 <sup>a</sup>	27.00±2.34 <sup>a</sup>	55.83±3.01 <sup>a</sup>
2%	40.83±7.18 <sup>a</sup>	26.33±0.45 <sup>a</sup>	59.30±8.49 <sup>a</sup>
4%	44.77±2.55 <sup>a</sup>	26.47±0.67 <sup>a</sup>	62.67±4.83 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

E. Texture profile analysis

The texture results are shown in Table VI. The cohesiveness did not differ significantly. The incorporation of the microalga caused the chewiness to decrease as the percentage increased, but it was the opposite for day 3. The microalgae may cause these results, since they lack a cell wall and are composed of a membrane made of sulfated polysaccharides (PS). Generally, the cell wall of algae comprises more than 40% of these. PS is widely used in the food industry to enhance and stabilize food structure. The negative charge of the PS interacts with cations present in food solvents such as milk or water, leading to gelation [18].

TABLE VI  
RESULTS OF TEXTURE PROFILE ANALYSIS

Treatments	Hardness (N)	Cohesiveness	Chewiness
Day 0			
Control	122.00±52.10 <sup>a</sup>	0.87±0.03 <sup>a</sup>	9973±403 <sup>a</sup>
2%	38.20±4.70 <sup>b</sup>	0.90±0.01 <sup>a</sup>	3200±346 <sup>b</sup>
4%	28.12±9.91 <sup>b</sup>	0.89±0.00 <sup>a</sup>	2317±841 <sup>b</sup>
Day 3			
Control	27.30±18.30 <sup>a</sup>	0.90±0.02 <sup>a</sup>	1202±94 <sup>b</sup>
2%	11.36±4.90 <sup>a</sup>	0.91±0.02 <sup>a</sup>	1484±251 <sup>b</sup>
4%	37.40±17.00 <sup>a</sup>	0.84±0.07 <sup>a</sup>	3662±95 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

F. Sensory evaluation

Table VII shows the results of the sensory evaluation, which showed no significant differences among treatments for the evaluated variables. This indicated that the 4% *Porphyridium cruentum* treatment was well received by the panelists. A low level of incorporation into food was better accepted due to the strong flavor of the dried microalgae. Still, significant additions tend to modify the rheological and technological properties of the original matrix [19].

TABLE VII  
RESULTS OF THE SENSORY EVALUATION OF THE PANCAKES

Value	Control	2%	4%
Smell	5.24±1.20 <sup>a</sup>	4.96±1.69 <sup>a</sup>	5.44±1.12 <sup>a</sup>
Color	3.92±1.32 <sup>a</sup>	4.24±1.53 <sup>a</sup>	4.24±1.64 <sup>a</sup>
Flavor	4.36±1.19 <sup>a</sup>	4.80±1.50 <sup>a</sup>	5.28±1.40 <sup>a</sup>
Hardness	4.20±1.26 <sup>a</sup>	4.24±1.61 <sup>a</sup>	4.72±1.51 <sup>a</sup>
Overall acceptability	4.44±0.96 <sup>a</sup>	4.32±1.49 <sup>a</sup>	4.68±1.41 <sup>a</sup>

\* Different letters in the same parameter show a significant difference.

V. CONCLUSIONS

The percentage of protein, ashes, and pH in the pancakes increased, and the sensory evaluation showed no significant differences; acceptance was obtained with the addition of 4% *Porphyridium cruentum*.

ACKNOWLEDGMENT

The author is thankful to the Faculty of Agronomy and the Environmental Remediation Laboratory of the Autonomous University of Nuevo León for the use of their facilities and the facilities granted.

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