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Microalgae Incorporated in Bakery Products: Application to Millet Pretzel

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Authors' contributions

This work was carried out in collaboration among all authors. Author AKM designed the research, carried out the research, analysed the data, wrote and revised the article. Author AL supervised research progress, reviewed the article, and approved the submission. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study was to develop and physico-chemical analysis for a recipe for millet pretzels by using two different blends of foxtail millet and little millet flour, incorporated with *Chlorella vulgaris* microalgae biomass at varying concentrations of 0.6%, 0.8% and 1.0% (measured as grams per 100 grams of flour). For the pretzel samples labeled CW0.6, CW0.8 and CW1.0, the flour base consisted of 80% foxtail millet flour and 20% little millet flour. On the other hand, the samples labeled TS0.6, TS0.8 and TS1.0 were made using a base of 75% foxtail millet flour and 25% little millet flour. The research conducted a thorough analysis to understand how these different flour blends and the addition of microalgae impacted the physico - chemical properties of the final products. Additionally, the sensory quality was assessed by a semi-trained panelist based on color, appearance, taste, flavor, texture and overall quality. The study revealed that the combination of different flour blends and varying concentrations of microalgae had a

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significant impact on the protein content of the pretzels. Specifically, pretzels with 1% microalgae biomass stood out due to their high protein content, making them a nutritious option. However, pretzels formulated with 0.8% microalgae received higher scores in sensory evaluations, indicating that this concentration was more favorable in terms of taste and overall sensory experience. In terms of other physico - chemical properties, the moisture content of the pretzels ranged from 31.67% to 33.95%, and the ash content varied from 1.653% to 1.953%. The findings of this study suggest that it is feasible to produce millet pretzels incorporated with microalgae that are both nutritious and appealing to consumers. This research opens up new possibilities for developing healthy and tasty snack options using millet and microalgae.

Keywords: Millet; foxtail; little; microalgae; pretzel; sensory; protein.

1. INTRODUCTION

The growing world population has led us to the hunger problem, which affects 1 out of 9 people in the world. The key challenge that we are going to face in this 21st century is the distribution and fulfillment of food requirements for this increasing human population while we have a limited amount of food sources [1]. The current amount of food production to feed people needs to be doubled to meet the demand of the expected world population by the year 2050 which is estimated at 9.8 billion [2]. The exponential rise of the world population requires new sources of sustainable protein supply because the majority of individual are lacking of appropriate calorie and protein intake required for their nourishment. The problems become worse as the world is not ready enough to find necessary solutions [1]. Another supply of protein-rich food is animal-based protein; however, the growing concerns about the welfare of animals as well as new food trends have lowered the supply of it [2].

Besides that, our agriculture system is affected by harsh climate change. The soil is reducing its nutrients and the underground water level is depleting. It leads to a crisis in the agriculture supply chain also which has a significant effect on the global food supply chain. Therefore, a search for new food sources is very much needed as it is the only way to tackle the current hunger problem with this rising population. The Sustainability and supply of healthy food can have a significant role in this.

Microalgae offer a reliable and promising solution for creating a sustainable and innovative food supply chain that addresses the problem of protein malnutrition [3]. Microalgae have an ancient lineage dating back 3.4 billion years, as evidenced by the fossilization of the oldest known microalga, a cyanobacteria, in rocks found in Western Australia [4]. Microalgae are distributed worldwide, predominantly inhabiting aquatic ecosystems but also present in terrestrial environments. They can be found as single entities or in symbiotic relationships with other organisms. Chlorella is a freshwater single-celled green alga. It comprises approximately 55-60% protein. Among the numerous species identified, *Chlorella vulgaris* has been extensively researched. The first scientifically pure algal culture was *Chlorella vulgaris*, was cultivated by Dutch microbiologist M.J. Beijerinck in 1890. Over years of intensive study on microalgae, researchers worldwide have speculated that their high nutritional value suggests the potential for large-scale production, potentially revolutionizing agriculture in the future [5]. Since ancient times, it has been used as an alternative medicine in the Far East and is recognized as a traditional food in the Orient. Renowned as a promising source of nutrients such as carotenoids and protein, it is widely produced and distributed as a dietary supplement in Japan, China, the U.S., and Europe. However, it is commonly believed that it does not have Generally Recognized as Safe (GRAS) status [6]. *Chlorella vulgaris* is notable among the limited selection of microalgae available in the market for use as a food supplement. Various flour compositions have been utilized to harness the potential of microalgae [4]. Incorporation of microalgae in a blend of millet flour can lead a new food product as a replacement of refined wheat flour. It can open a new category for healthy millet based baked product. Microalgae biomass can offer several benefits to human nutrition, including proteins and minerals. In this sense, microalgae-based foods are already evident as opportunities for food research and development. Furthermore, the production of microalgae brings advantages to global food security and reduces the impact considering environmental problems related to the expansion of food production in agricultural land.

The research study is aimed to create pretzels using a combination of millets and microalgae. Pretzel is a type of baked bread product typically shaped into a twisted knot. It is originated in Europe during the Early Middle Ages, possibly among monks. The traditional pretzel has a distinctive non-symmetrical form, with the ends of a long strip of dough intertwined and then twisted back on it. This study evaluated the effect of millet flour and the incorporation of microalgae on the physico - chemical properties of the pretzels, as well as conducted a sensory evaluation of the final product.

2. MATERIALS AND METHODS

2.1 Materials

The study material included dough of millet pretzel prepared using two blends of foxtail millet and little millet flour. Microalgae *Chlorella vulgaris* powder (Source: Amedeo Ventures Private Limited, Maharashtra, India) was added to the dough at three different concentrations: 0.6, 0.8 and 1.0% (g/100 g flour). In samples marked CW0.6, CW0.8 and CW1.0, the base was a blend of foxtail millet flour (80%) and little millet flour (20%), while in TS0.6, TS0.8 and TS1.0, the base was a blend of foxtail millet flour (75%) and little millet flour (25%).

The amounts of all ingredients in the dough recipe were expressed in % (g/100 g flour) and were the same in all samples: 18.03% of tapioca starch, 10.02% of corn starch, 0.67% of xanthan gum, 4.01% of psyllium husk, 4.01% of butter, 4.21% of sugar, 1.36% of table salt and 1.6% of instant dry yeast. These materials were purchased from local market of Prayagraj.

2.2 Dough Preparation and Baking

The pretzel was prepared at laboratory of Department of Processing and Food Engineering by following the procedure reported by Jan et al., (2021); Haroon et al., (2007) with minor modification [7,8].

Firstly, proofing of yeast was done by adding instant dry yeast in sugar mixed lukewarm water and allowed to sit for 10 minutes. Meanwhile, psyllium husk and xanthan gum were mixed with 60 ml lukewarm water to make gel. Standard dry ingredient mixture was prepared by mixing flours, starches, sugar, salt and microalgae. Then all these were combined to make dough with addition of required potable water. After kneading of dough, it was given rest for 70 minutes to raise the volume double of its previous weight. Then the shape was given to make pretzel. Then the pretzel was given hot water-baking soda bath for 30 seconds. Baking was done at 200 -210℃ for 40-45 minutes.

2.3 Pretzel Analysis

2.3.1 Moisture content

Moisture content of the sample was determined using the method described by Khemiri et al. [9]. To determine the moisture content, 5g of the sample was taken in the aluminium petri dish, dried in a hot air oven at 130±2°C, then cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was achieved. The percentage moisture was calculated as follows:

$$\text{Moisture (\%)} = \frac{\text{Moisture loss in g}}{\text{Mass in g of sample}} \times 100$$

2.3.2 Ash content

Ash content was determined as described in by Khemiri et al. [9]. To determine the ash content, 5g of sample was taken into the crucible of known weight, which was dried and kept in a muffle furnace at 550±25°C till grey ash was obtained. The ash percentage was calculated as follows:

$$\text{Total ash on dry basis (\% by weight)} = \frac{\text{Ash content in g}}{\text{Mass of sample in g}} \times 100$$

2.3.3 Protein content

Crude protein was determined using the micro-kjeldahl method as mentioned in by Khemiri et al. [9]. In a dry and clean Kjeldahl flask, 1g of sample was properly mixed with 5g Potassium sulfate, 3g of the copper sulphate and 20 ml of concentrated sulfuric acid. The whole solution was digested for at least 150 minute until the digest was clear and free of undigested material. After the digest was cooled to 25°C, 100 ml of distilled water was added to Kjeldahl flask. After cooling the solution at 25°C, 75 ml of 40% (m/m) sodium hydroxide solution was added to the diluted digest by carefully pouring the solution down the inclined neck of the Kjeldahl flask. Immediately after the addition of sodium hydroxide solution to the Kjeldahl flask, it was connected to the distillation apparatus, the tip of whose condenser outlet tube was immersed in 50 ml of 40% (m/m) boric acid solution with indicator contained in a 500 ml Erlenmeyer flask. The receiving boric acid solution was titrated with standard hydrochloric acid solution (0.1 N) to the first trace of pink colour.

The crude protein was calculated by multiplying Nitrogen by the conversion factor of 6.25.

Nitrogen Content in Sample (% by Mass) =

$$\frac{\text{HCl used for sample in ml} - \text{HCl used for blank in ml}}{\text{Mass of test portion in g}} \times 1.4007 \times \text{Normality of HCl}$$

2.4 Sensory Evaluation

Pretzel samples with microalgae biomass, as well as the control sample, were tested by a semi-trained sensory analysis panel selected among faculties and students of Sam Higginbottom University of Agriculture, Technology and Sciences. Among the respondents, 55% were women and 45% were men. The age distribution of the respondents was as follows: 60% were in the 18–25 age range, while 40% were in the 28–50 age range.

The participants were asked to rate various quality characteristics on a 9-point hedonic scale (1= dislike extremely; 2=dislike very much; 3=dislike moderately; 4=dislike slightly; 5=neither like nor dislike; 6=like slightly; 7=like moderately; 8=like very much; 9= like extremely). The quality characteristics assessed were as follows: color, flavor, taste, texture, and overall acceptance.

2.5 Statistical Analysis

The obtained results were analyzed using R Studio software from Posit PBC (version RStudio-2024.04.1-748), through variance analysis (one-way ANOVA). The level of significance was set at *P*= 0.05. The results of analyses were illustrated as mean.

3. RESULTS AND DISCUSSION

3.1 Moisture Content

The replacement of millet flour composition did not significantly affect the moisture content of the pretzel if comparing all the treatments. The addition of microalgae led to a significant reduction in moisture content across the samples (Table 1). The highest moisture content was recorded for sample (TS0.8) with 33.95% and lowest found in sample (CW1.0) with 31.67%. Increasing the microalgae biomass concentration led to decreasing the moisture content. Marzec et al., (2023) summarized similar findings that the addition of *Chlorella vulgaris* led to a significant reduction ($p < 0.001$) in moisture content while working with muffins. They found that specifically the addition of 0.5% microalgae resulted in decrease in moisture content of about 1 p.p. (percentage point) and 1.5% microalgae reduced the moisture content by about 3 p.p. compared to samples without microalgae [10]. Similar result was also found by Batista et al., (2019) when they had increased the concentration of *Chlorella vulgaris* from 2% to 6% while making crackers [11]. Dündar et al., (2023) also showed that moisture was decreased from 5.86% to 5.11% when they had increased the concentration of *Chlorella vulgaris* from 0.5% to 3% in cookies [12].

3.2 Ash Content

According to Table 2 ash content was increased with different composition of millet where composition of foxtail millet was increased. Also the ash content was also increased with the increased concentration in *Chlorella vulgaris*. This is because millets are rich in micronutrients like calcium, minerals etc. Beside that *Chlorella vulgaris* is also rich in micronutrients. The highest ash content was recorded for sample (TS1.0) with 1.953% and lowest found in sample (CW0.8) with 1.653%. Similar result was also found by Passi et al., (2023) where they had concluded that ash content was significantly increased with increased substitution levels of foxtail millet flour in bread [13]. Widyaningrum & Prianto (2021) reviewed many papers and concluded that addition of microalgae increased the ash content [14]. Dündar et al., (2023) also concluded that ash content was increased from 0.49% to 0.55% when they had increased the concentration of *Chlorella vulgaris* from 0.5% to 3% in cookies made of refined wheat flour [12].

3.3 Protein Content

According to Table 3 protein content was increased with the increased concentration in *Chlorella vulgaris*. This is because of *Chlorella vulgaris* which has high content of protein i.e. 60% as mentioned by Rani et al., [5]. The highest protein content was recorded for sample (TS0.8) with 1.930% and lowest found in sample (CW0.6) with 1.758%. Batista et al., (2019) verified in their formulation of cracker that crackers with microalgae found with highest values of protein content compared with control sample [11].

Table 1. Moisture content of millet pretzel

Treatment	Mean (%)
CW0.6	33.63
CW0.8	33.57
CW1.0	31.67
TS0.6	33.73
TS0.8	33.95
TS1.0	32.28
F Statistic	
F-Value	257.2089
Fcritical	2.7728

Table 2. Ash content of millet pretzel

Treatment	Mean (%)
CW0.6	1.653
CW0.8	1.763
CW1.0	1.913
TS0.6	1.727
TS0.8	1.880
TS1.0	1.953
F Statistic	
F-Value	13.902
Fcritical	2.7728

Table 3. Protein content of millet pretzel

Treatment	Mean (%)
CW0.6	1.758
CW0.8	1.780
CW1.0	1.914
TS0.6	1.886
TS0.8	1.875
TS1.0	1.930
F Statistic	
F-Value	69.1778
Fcritical	2.7728

In fact, microalgae-enriched pretzel can be regarded as a very interesting protein-fortified bakery product. Similar result also found in Widyaningrum & Prianto (2021, July) review paper where they had showed that addition of microalgae increased the protein content [14]. Dündar et al., (2023) also concluded increment of protein content with the increased concentration of *Chlorella vulgaris* from 0.5% to 3% in cookies [12].

3.4 Sensory Evaluation

After development of Microalgae incorporated Millet Pretzel sensory evaluation was done by semi-trained panel members. Fig. 1 presents the summary of the results from the evaluation of sensory characteristics, providing the mean values of all attributes assessed.

According to Fig. 1 all the samples scored a good acceptance. But TS0.8 was the most favorable to panelist as it got highest score in taste and overall acceptability. However, it is worth mentioning that the green color did not play any negative effect in appearance for all samples.

In the formulation of cracker with refined wheat flour Batista et al., (2019) concluded that addition of microalgae attained low sensory scores mainly because of their unattractive fishy off-flavor [11]. But in this present study, microalgae incorporation in millet flour had shown no such flavor as no sensory panel members had termed such flavor in sensory evaluation. All samples had minimal differences in flavor score. Thus it was concluded that the fishy off-flavor is neutralized by millet flour.

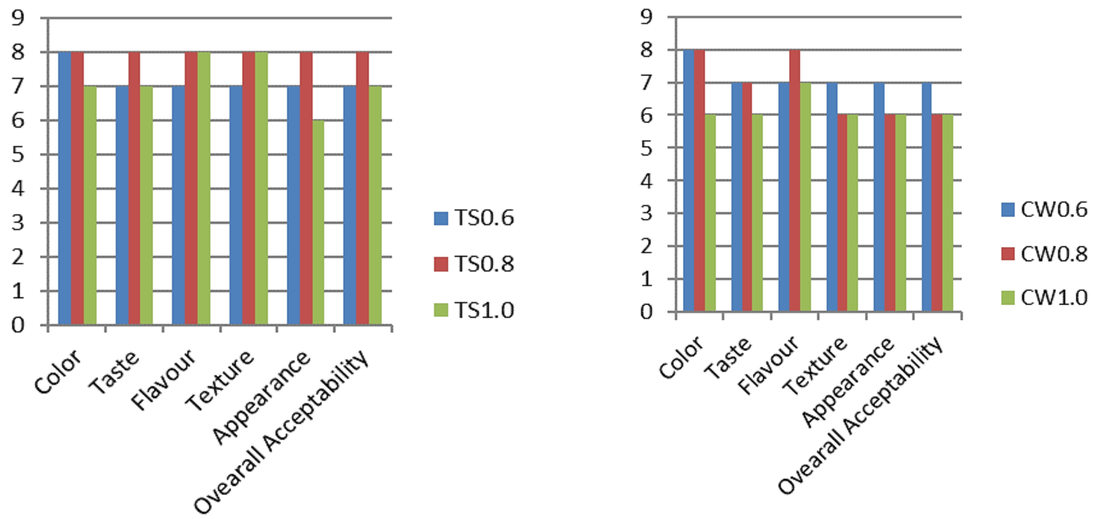

Fig. 1. Sensory evaluation of millet pretzel. Explanations: CW- foxtail millet flour (80%) and little flour (20%), TS- foxtail millet flour (75%) and wheat flour (25%)

4. CONCLUSION

In this study, microalgae biomass was successfully incorporated into a blend of foxtail millet flour and little millet flour to prepare a recipe for millet pretzels. The physico – chemical analysis of all samples lightened that addition of microalgae led to decrease in moisture content and increase in ash content. Particularly, pretzels with 1.0% *Chlorella vulgaris* biomass were notable for their high protein content, qualifying them for a "source of protein" claim. The microalgae pretzels gave an attractive green color that got a positive response by sensory analysis panelists. However, pretzels with 0.8% microalgae formulation received higher sensory analysis scores. Although the microalgae strains *Chlorella vulgaris* has high protein content, but their low sensory scores delay their widespread use. Additionally, further research work is needed to confirm the nutritional value of these newly developed millet pretzels.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Torres-Tiji Y, Fields FJ, Mayfield SP. Microalgae as a future food source. Biotechnology Advances. 2020;41:107536.
2. Caporgno MP, Mathys A. Trends in microalgae incorporation into innovative food products with potential health benefits. Frontiers Innutrition. 2018;5:58.
3. Maehle N, Skjeret F. Microalgae-based food: Purchase intentions and willingness to pay. Future Foods. 2022;6:100205.
4. Safi C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. Renewable and Sustainable Energy Reviews. 2014; 35:265-278.
5. Rani K, Sandal N, Sahoo PK. A comprehensive review on chlorella-its composition, health benefits, market and regulatory scenario. The Pharma Innovation Journal. 2018;7(7),584-589.
6. Gouveia L, Batista AP, Miranda A, Empis J, Raymundo A. *Chlorella vulgaris* biomass used as colouring source in traditional butter cookies. Innovative Food Science &

Emerging Technologies. 2007;8(3):433-436.
7. Jan N, Naik HR, Gani G, Bashir O, Hussain SZ, Rather AH, Amin T. Optimization of process for the development of rice flour incorporated low-gluten wheat based pretzels: Evaluation of its physicochemical, thermal and textural characteristics. Journal of the Saudi Society of Agricultural Sciences. 2021; 20(2):116-127.
8. Haroon RN, Sekhon KS, Baljit S, Amarjeet Kaur AK, Rather AH. Influence of triticales and wheat classes on pretzel quality; 2007.
9. Khemiri S, Khelifi N, Nunes MC, Ferreira A, Gouveia L, Smaali I, Raymundo A. Microalgae biomass as an additional ingredient of gluten-free bread: Dough rheology, texture quality and nutritional properties. Algal Research. 2020;50: 101998.
10. Marzec A, Kramarczuk P, Kowalska H, Kowalska J. Effect of type of flour and microalgae (*Chlorella vulgaris*) on the rheological, microstructural, textural, and sensory properties of vegan muffins. Applied Sciences. 2023;13(13):7632.
11. Batista AP, Niccolai A, Bursic I, Sousa I, Raymundo A, Rodolfi L, Tredici MR. Microalgae as functional ingredients in savory food products: Application to wheat crackers. Foods. 2019;8(12):611.
12. Dündar AN, Sahin OI, Saricaoglu FT. Low-fat cookies with *Chlorella vulgaris*: Effects on dough rheology, physical, textural and sensory properties of cookies. Gida. 2023; 48(3):526-544.
13. Passi P, Kaur K, Kaur J, Kaul S. Quality characterization, bread making properties and storage stability of foxtail millet incorporated bread ready-mix. Journal of Stored Products Research. 2023;104: 102208.
14. Widyaningrum D, Prianto AD. Chlorella as a source of functional food ingredients: Short review. In IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2021, July;794(1):012148.

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