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Assessment of Sensory Attributes and Nutritional Profile of Microalgae Nuggets Derived from *Chlorella vulgaris*

Sona A*, Asiya Khan MS, Veena Viswanath, Priya Senan V

SAS SNDP Yogam College, Konni, Pathanamthitta, Kerala, India.

* Corresponding author email: bijusona442@gmail.com

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ABSTRACT

This study explored the use of *Chlorella vulgaris*, a microalgae strain, in nugget production, comparing it to traditional chicken nuggets and a vegetarian control sample. The microalgae culture showed gradual growth, reaching its peak on the 15th day of inoculation. Microscopic examination revealed both single cells and colonies of *Chlorella vulgaris*. DNA isolation, sequencing and nucleotide blast was performed to confirm the cultured species. Optical density measurements at 700nm and chlorophyll content analysis provided insight into growth dynamics. Cell counting was performed under a light microscope for precise cell density calculations. Sensory evaluation, conducted by a panel of fifteen individuals aged 19 to 26, assessed attributes like color, aroma, crispiness, taste, and overall acceptance using a nine-point hedonic scale. Proximate composition analysis unveiled noteworthy differences among nugget samples. Microalgae nuggets (F2) had higher moisture, ash, and crude protein content compared to chicken nuggets (F1) and the control sample (F3). F2 also contained less crude fat but significantly more protein than F1 and F3. F2 is superior to F1 and F3 on sensory evaluation. This study underscores *Chlorella vulgaris* potential as a sustainable and nutritious ingredient in nugget production, offering alternatives for protein sourcing.

Key words : Sensory Evaluation, Proximate Composition, Chlorophyll Content, Nugget Production, Novel Food Products

INTRODUCTION

In an era marked by a growing global population, concerns over food security, and escalating environmental challenges, innovative approaches to food production have gained paramount significance. The quest for alternative protein sources and sustainable food options has intensified as traditional agricultural practices strain the earth's resources and exacerbate environmental degradation. Microalgae, recognized as a sustainable biological resource, possess a well-balanced biochemical composition characterized by high protein content and the presence of bioactive compounds like carotenoids and essential fatty acids, offering potential health benefits (Lucas *et.al.*, 2018). However, despite the vast diversity of identified microalgal strains, only a limited selection has obtained approval for human consumption. European Union has approved *Chlorella vulgaris* as safe usage and is

present in the market even before 15th May 1997 (EU Novel Food Catalogue). Microalgae, with their remarkable nutritional value and sustainable cultivation characteristics, have emerged as a promising solution to address these multifaceted issues. Among these microorganisms, *Chlorella vulgaris* stands out as a versatile and nutritionally rich candidate.

Chlorella vulgaris is a versatile microalga known for its nutritional richness, photosynthetic capabilities, and potential health benefits, making it a notable player in the fields of nutrition, biotechnology, and sustainable agriculture (Fazal *et.al*., 2021). Its composition on a dry basis comprises of 20% carbohydrates, 5% fiber, 10% minerals and vitamins,20% fat namely poly unsaturated fatty acids, and a remarkable 45% protein. Furthermore, it boosts the presence of beneficial carotenoids such as β-carotene, lutein, and zeaxanthin. This remarkable combination of essential nutrients and antioxidants makes *Chlorella vulgaris* a valuable addition to a balanced diet. *Chlorella vulgaris* finds wide applications in production of primary and secondary metabolites, for generation of biodiesel and biogas, for bioremediation, and in cosmetology apart from use as supplementary food (Coronado-Reyes *et.al*., 2020).

This study embarks on a pioneering journey to explore the development of microalgae nuggets using *Chlorella vulgaris*, presenting a holistic examination of their sensory attributes and nutritional profile.The study transcends the mere cultivation and harvesting of *Chlorella vulgaris*; it delves into the innovative transformation of these microalgae into nugget formulations-a promising avenue for integrating microalgae into our daily diets. The research endeavours to decipher the sensory characteristics of microalgae nuggets, evaluating aspects such as texture, color, aroma, and taste. Simultaneously, an in-depth nutritional analysis will unveil the potential health benefits and dietary advantages of incorporating *Chlorella vulgaris* into such innovative food products.

In a world grappling with the paradox of food abundance coexisting with malnutrition and ecological crises, this investigation sheds light on a novel frontier in food science. The development of microalgae nuggets represents a potential breakthrough that not only addresses pressing global issues but also offers a palatable and nutritious solution. This comprehensive analysis aims to bridge the gap between sustainability, nutrition, and sensory appeal, offering insights into the future of sustainable food production and consumption.

The primary objective of this study is to comprehensively investigate the growth, characterization, and potential applications of a specific strain of *Chlorella vulgaris* microalgae. This research aims to evaluate the optimum growth kinetics and biomass production of *Chlorella vulgaris* under controlled culture conditions. The microalgal species identification using rRNA sequencing and BLAST. *Chlorella vulgaris* nuggets were prepared and compared with chicken nuggets and control. The sensory evaluation and determination of proximate chemical composition of the novel food product were carried out to assess their acceptability and nutritional attributes. This study aims to contribute valuable insights into the cultivation and utilization ofmicroalgae,

with potential implications for various biotechnological and nutritional applications.

Materials and Methods

Microalgal Culture : Microalgal species selected for the current study was a strain of *Chlorella vulgaris* which was previously isolated and preserved in Scire Science R & D laboratory, KINFRA Kalamassery, Kerala, India.

Culture Media, Composition and Parameters : The algae culture broth was prepared and the culture tubes were incubated under optimum conditions as working volume 1 litre, temperature 24±1ºC, salinity 13-15%, light intensity 700-800 lux, photo period 16/18h, for 15 days.

Microscopic Observation : After 5 days of incubation, the culture was observed microscopically under 40X magnification using a light microscope (ZEISS primo star) on a daily basis in order to examine growth and multiplication of Microalgal cells.

Maximum Absorbance : The optical absorbance was measured at the wavelength of 700nm using spectro-photometer (LAB India).

Cell Counting : Microalgae concentration was measured using a Neubauer haemocytometer, and cell density (cells/mL) was calculated.

Determination of Chlorophyll Content : Chlorophyll was extracted from the microalgal pellet using 90% methanol and measured for absorbance at 450nm after blanking the UV spectrophotometer with acetone.

Identification of Microalgae using Molecular Sequencing : Microalgal DNA was isolated using the method described by Doyle and Doyle, 1987. DNA yield and purity were assessed using a UV spectrophotometer (260nm/ 280nm ratio) and by running samples on a 0.1% agarose gel with a 250bp DNA marker, visualized using a BIORAD Molecular Image system. The 18s region was PCR-amplified (20µl reaction with 20ng template DNA and specific primers), followed by 40 cycles of denaturation, annealing, and extension. Amplicons were gel-checked, purified, and sequenced using ABI 3730 XL. The obtained sequence was subjected to a comparative analysis using the BLAST bioinformatics tool, which involved a search for similarities with sequencesstored in the NCBI database.

Nugget preparation : Raw materials, including yam, carrot, and chicken were sources from local areas and markets in Kottayam, Ernakulam and Kakkanad for nugget preparation.For algal biomass preparation, *Chlorella vulgaris* was harvested, lyophilized and stored at -20°C. Then the lyophilized *Chlorella vulgaris* were

Table 1 : Ingredients used for preparation of nuggets

F1	F2	F3
250g of chicken nuggets boneless chicken was minced	250g yam and 100g carrot was boiled and minced	
mixed with ginger-garlic paste, chili powder, black pepper, veg masala powder, a pinch of salt		
-	1g of microalgae added	-
Marinated for 30-minutes	Refrigerated for 15 minutes	
The dough was shaped into nuggets, dipped them in corn flour solution, coated with breadcrumbs, and deep-fried.		

Sensory Evaluation : Sensory evaluation assays of nuggets samples(F1, F2, F3) were performed at Scire Science R&D laboratory, KINFRA, Kalamassery, Kerala, India. A panel of 15 members, mostly students and staff age ranges between 19 to 26 were chosen for the sensory evaluation and attributes evaluated were texture, colour, aroma, astringency or taste and overall acceptance. A nine-point hedonic scale was used as adopted by Sabikun et al., 2020.

Proximate Chemical Composition Determination : Ash content, moisture content, crude protein content, crude fibre, fat contents were determined by using AOAC method, 2002. Carbohydrate content was determined by the FAO difference method.

Results and Discussion

On the first day of inoculation; the Microalgal culture had a slight yellowish green colour (Fig:1). The culture reached its maximum growth on the 15th day of inoculation (Figure 2).



Figure 1



Figure 2

Figure 1 and 2 represents the microalgal culture on the first and 15th day of inoculation respectively. Figure 3 represents the microscopic image of Chlorella vulgaris under 45X magnification. Both single cells and colonies were counted using a haemocytometer.

Maximum absorbance and Chlorophyll content was maximum on Day 15 (fig 4) and hence the cell count determined using hemocytometer (fig 5). *Chlorella* is an algae that is high in protein content, vitamins, minerals and exceptionally high chlorophyll content making it a commercial source (Tang & Suter, 2011).

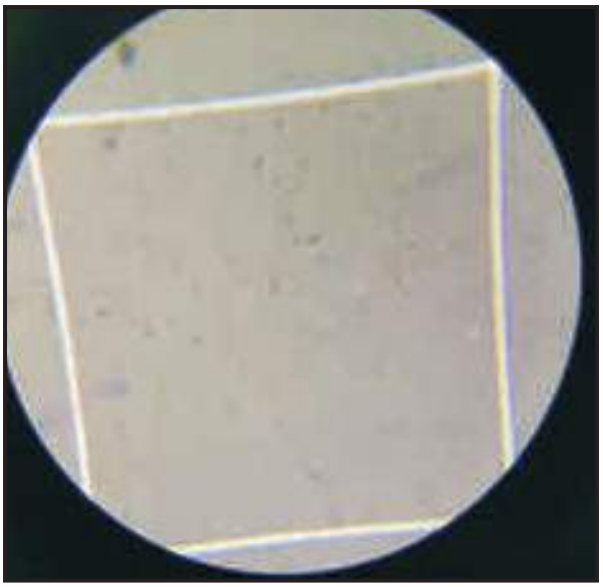


Figure 3 : Microscopic observation of *Chlorella vulgaris* under 45X magnification

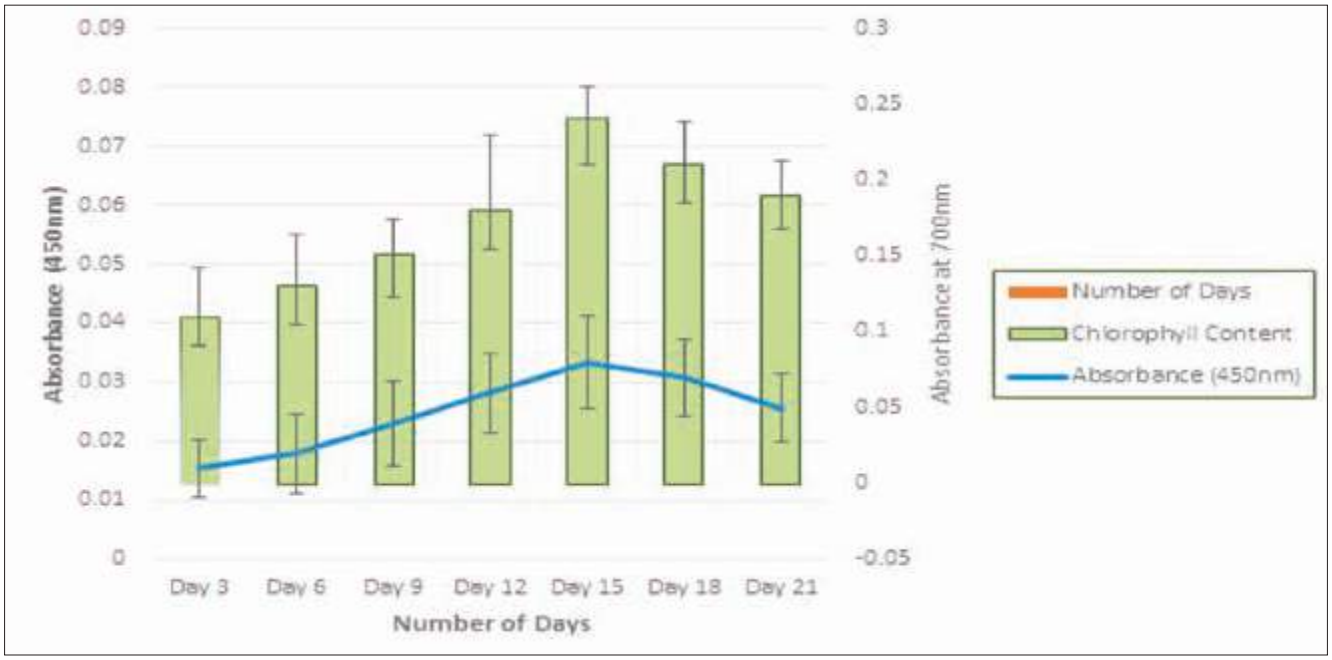


Figure 4: Determination of Maximum Absorbance (Culture) and Chlorophyll content

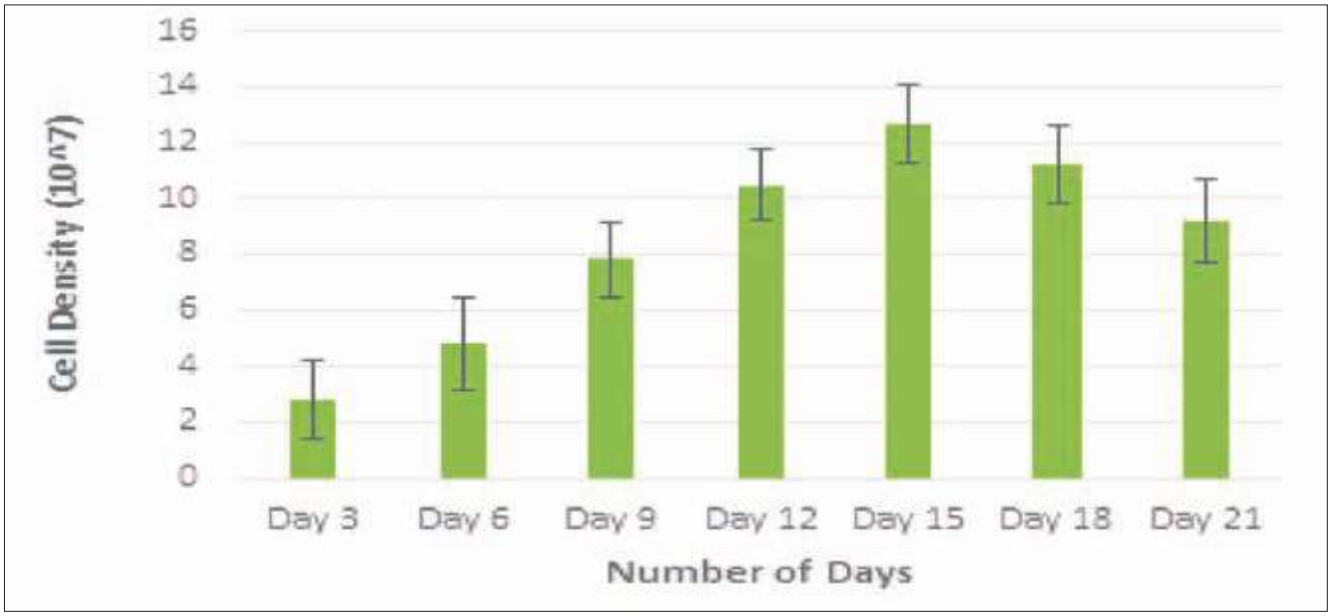


Figure 5 : Determination of Cell Density

Figure 6 shows the gel image of DNA isolated using CTAB method followed by the 18S rRNA sequence and BLAST (fig7) and phylogenetic tree (fig 8). Results prove that the cultured microalgae are *Chlorella vulgaris*.



Figure 6 : Ethidium bromide stained 2% agarose gel showing 18S rRNA PCR amplification of DNA. Lane (MBTO51): 100 bp marker. Lane (S): 18S rRNA PCR amplicon of DNA.

Molecular Identification of Microalgae

>AF350260.1:1-586 Chlorella vulgaris 18S ribosomal RNA gene, partial sequence
AGGGACAACCATTGGAAACGATGGCTAA
TACCTCATAATACTGAGTAAGTTAAATGAT
GAATAATCGCCAAGAGATGGGCTTGCGG
CTGATTAGCTAGTTGGTGGGGTAAAGGC
CTACCAAGGCGATGATCAGTATCTGGTC
TGACCAGGATGATCACCCACACTGGGA
CTGAGACACGGCCCAGACTCCTACGGG
AGGCAACAGTGAGGAATTTTCCGCAATG
GGCGAAAGCCTGACGGAGCAATGCCGC
GTGAAGGATGAAGGCCTATGGGTTGTAA
ACTTCTTTTCTCAGAGAAGAAATTTTGAC
GGTATCTGAGGAATAAGCATCGGCTAAC
TCTGTGCCAGCAGCCGCGGTAAGACAG
AGGATGCAAGCGTTATCCGGAATGATTG
GGCGTAAAGCGTCTGTAGGTGGCTTAAA
AAGTCTCCTGTCAAAGATCAGGGCTTAA
CCCTGGGCCGGCAGGAGAAACTCTTAG
GCTAGAGTTTGGTAGGGGCAGAGGGAA
TTCCCGGTGGAGCGGTGAAATGCGTAG
AGATCGGGAGGAACACCAAAGGCGAAA
GCACTCTGCTGGGCCACAACTGACACT
GAGA

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chlorella vulgaris 16S ribosomal RNA gene, partial sequence; chloroplast gene for chloroplast product	Chlorella vulgaris	1083	1083	100%	0.0	100.00%	586	AF350260.1
Chlorella vulgaris strain MSU-AGM 14 16S ribosomal RNA gene, partial sequence; chloroplast	Chlorella vulgaris	1050	1050	100%	0.0	98.98%	609	KM189121.1
Chlorella sp. ArM0029B chloroplast, complete genome	Chlorella sp. Ar...	1042	1042	100%	0.0	98.81%	119989	KP554427.1
Micractinium singularis strain MM0003 plastid, complete genome	Micractinium sin...	1037	1037	100%	0.0	98.63%	139597	MN894267.1
Micractinium pusillum strain CCAP 232/1 chloroplast, complete genome	Micractinium pus...	1031	1031	100%	0.0	98.46%	115638	MN649872.1
Micractinium sp. LBA 32 chloroplast, complete genome	Micractinium sp....	1026	1026	100%	0.0	98.29%	109688	MH983006.1
Uncultured Streptophyta clone UVmas1_53 16S ribosomal RNA gene, partial sequence; chloroplast	uncultured Strep...	1026	1026	100%	0.0	98.29%	701	JQ701246.1
Uncultured bacterium clone AD05 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1024	1024	100%	0.0	98.29%	990	KC009751.1
Uncultured Streptophyta clone UV-2_3 16S ribosomal RNA gene, partial sequence; chloroplast	uncultured Strep...	1020	1020	100%	0.0	98.13%	706	JQ700677.1
Uncultured cyanobacterium clone Gap-2-18 16S ribosomal RNA gene, partial sequence	uncultured cyan...	1018	1018	100%	0.0	98.12%	870	EU642172.1
Chlorella sp. SUN-2 16S ribosomal RNA gene, partial sequence; plastid	Chlorella sp. SU...	1014	1014	100%	0.0	97.96%	1454	EF114678.1
Auxenochlorella pyrenoidosa isolate FACHB-5 chloroplast, complete genome	Auxenochlorella,...	1009	1009	100%	0.0	97.78%	107442	MN128434.1
Uncultured phototrophic eukaryote clone NV1_CYA_1_29 16S ribosomal RNA gene, partial sequence; plastid	uncultured photo...	1007	1007	100%	0.0	97.78%	666	FJ204892.1
Uncultured bacterium clone CK-86 16S ribosomal RNA gene, partial sequence	uncultured bacte...	1003	1003	100%	0.0	97.61%	1453	KM200526.1
Uncultured bacterium gene for 16S ribosomal RNA, partial sequence, clone: Sa75_M_3	uncultured bacte...	1002	1002	100%	0.0	97.61%	1415	LC065717.1
Uncultured bacterium clone N06Jun-31 16S ribosomal RNA gene, partial sequence	uncultured bacte...	996	996	100%	0.0	97.44%	803	EU442896.1
Uncultured bacterium clone P1O-64 16S ribosomal RNA gene, partial sequence	uncultured bacte...	996	996	100%	0.0	97.44%	809	EU375419.1
Uncultured cyanobacterium clone XZNMC45 16S ribosomal RNA gene, partial sequence	uncultured cyan...	990	990	100%	0.0	97.27%	1371	EU750214.1
Chlorella variabilis clone D1025 chloroplast, complete genome	Chlorella variabilis	986	986	100%	0.0	97.10%	118106	MZ647689.1
Chlorella sp. ATCC 30562 plastid, complete genome	Chlorella sp. AT...	983	983	100%	0.0	97.10%	124881	KY929917.1
Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Hados.Water.Euk.c.3	uncultured bacte...	979	979	100%	0.0	96.93%	1402	AB355044.1
Chlorella variabilis strain YTU_ANTARCTIC.001 small subunit ribosomal RNA gene, partial sequence; chloropl...	Chlorella variabilis	974	974	100%	0.0	96.76%	1359	MN372092.1
Prochlorococcus sp. EGEMACC 6 isolate A 16S ribosomal RNA gene, partial sequence	Prochlorococcus...	974	974	100%	0.0	96.76%	975	JQ726701.1
uncultured bacterium partial 16S rRNA gene	uncultured bacte...	972	972	100%	0.0	96.76%	1412	LR636220.1
uncultured bacterium partial 16S rRNA gene	uncultured bacte...	972	972	100%	0.0	96.76%	1413	LR636755.1
Chlorella heliozoae plastid, complete genome	Chlorella heliozo...	972	972	100%	0.0	96.76%	124353	KY629616.1
Pseudochlorella pringsheimii 16S ribosomal RNA gene, partial sequence; tRNA-Ile and tRNA-Ala genes, com...	Pseudochlorella ...	970	970	100%	0.0	96.59%	4781	MF683077.1
Uncultured bacterium clone AG09 16S ribosomal RNA gene, partial sequence	uncultured bacte...	970	970	100%	0.0	96.59%	936	KC009731.1
Uncultured Chlorella clone BF 009 16S ribosomal RNA gene, partial sequence	uncultured Chlor...	970	970	100%	0.0	96.59%	1455	KC994689.1
Uncultured Chlorella clone RFLP band pattern 11 16S ribosomal RNA gene, partial sequence	uncultured Chlor...	970	970	100%	0.0	96.59%	1452	KC994673.1
Uncultured bacterium clone AF06 16S ribosomal RNA gene, partial sequence	uncultured bacte...	965	965	100%	0.0	96.42%	895	KC009761.1
Chlorella sorokiniana isolate 1230 chloroplast, complete genome	Chlorella sorokin...	961	961	100%	0.0	96.42%	109803	KJ742376.1
Chlorella sorokiniana chloroplast, complete genome	Chlorella sorokin...	961	961	100%	0.0	96.42%	109811	KJ397925.1
Chlorella sp. JC183 plastid partial 16S rRNA gene, strain JC183	Chlorella sp. JC...	957	957	100%	0.0	96.25%	1449	HF536585.1
C.sorokiniana plastid DNA small subunit (16S-like) ribosomal RNA	Chlorella sorokin...	957	957	100%	0.0	96.25%	1493	X65689.1
Uncultured bacterium clone LB532 16S ribosomal RNA gene, partial sequence	uncultured bacte...	955	955	100%	0.0	96.25%	1453	MG238532.1
Chlorellaceae sp. M7 16S ribosomal RNA gene, partial sequence; plastid	Chlorellaceae sp...	955	955	100%	0.0	96.25%	1175	HM573453.1
Uncultured cyanobacterium clone LPSB10 16S ribosomal RNA gene, partial sequence	uncultured cyan...	955	955	100%	0.0	96.25%	1321	FJ901770.1
Uncultured bacterium clone 3-1 16S ribosomal RNA gene, partial sequence	uncultured bacte...	953	953	100%	0.0	96.08%	1461	KX823759.1
Uncultured bacterium clone CK-111 16S ribosomal RNA gene, partial sequence	uncultured bacte...	953	953	100%	0.0	96.08%	1466	KM200540.1
Uncultured eukaryote clone Ke0219r46 16S ribosomal RNA gene, partial sequence; plastid	uncultured eukar...	953	953	100%	0.0	96.08%	610	GQ422918.1
Cyanobacterium G1 16S ribosomal RNA gene, partial sequence	cyanobacterium,...	950	950	100%	0.0	96.08%	798	KT156639.1
Uncultured bacterium clone PAS3_C01 16S ribosomal RNA gene, partial sequence	uncultured bacte...	948	948	100%	0.0	95.90%	721	DQ830612.1
Chlorella vulgaris strain UTEX259 plastid, complete genome	Chlorella vulgaris	946	946	100%	0.0	95.90%	176851	MK948102.1

Figure 7 : BLAST results of isolated DNA sequence



Figure 8: Phylogenetic Tree

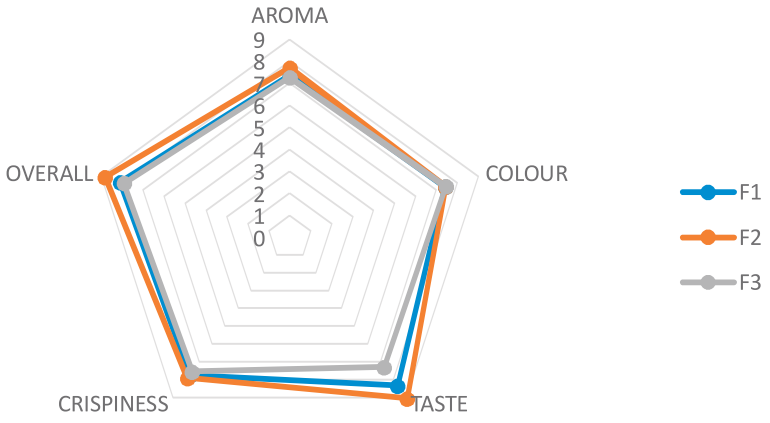


Figure 9 : Sensory Analysis

Figure 9 represents the average scores of the sensorial parameters as evaluated by the panel. Microalgae nuggets F2 were superior to F1 and F3 in smell, taste, crispiness and the overall contributions. Numerous studies have consistently demonstrated that sensory evaluations of microalgae-based products, including pasta Fradigue *et.al. 2010)*, cookies (Uribe - Wandurraga *et.al.* 2021), (yogurt Cho *et.al*, 2004) and cheese (Lousada Falcao *et.al*, 2023) consistently show positive consumer appreciation. Research indicates that 2% *Chlorella vulgaris. c*ookies are preferred for colour over 6% with no significant textural differences observed (Batista *et.al*, 2017). F1, F2 and F3 in figure 10 represents preparation of chicken nuggets, microalgal nuggets and control respectively.

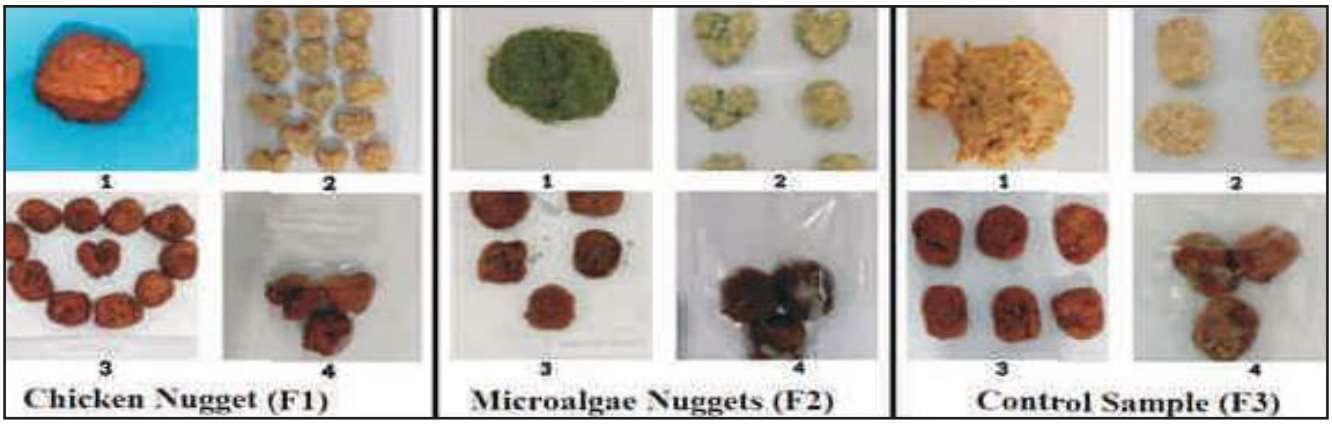


Figure : 10 Preparation of Chicken nugget (F1), Microalgae Nuggets (F2) and Control sample (F3) images

Table 2 : Proximate Composition of Nuggets

Sample Code	Moisture content (g/100g)	Total ash content (g/100g)	Crude protein content (g/100g)	Crude fat (g/100g)
F1	48.9±1.56	1.9±1.33	15±0.63	12.2±0.36
F2	49.1±2.34	2.0±0.94	17.1±0.53	11.1±0.09
F3	47.9±2.48	1.9±0.83	12.1±0.74	10.7±0.35

The proximate composition of F1, F2 and F3 nuggets substituted with chicken, microalgae and great yam are shown in table 2. Significant differences were observed in the fat, protein and ash content amongst the various nuggets. Moisture content was highest in F2sample (microalgae nugget) and having the lowest moisture content in the F3 Sample. The microalgal nugget have also high ash content and crude protein content than other samples. Crude fat content was comparatively lower than chicken nugget. Proximate composition of 2% *Chlorella vulgaris* cookies gave moisture content 3.2 ± 0.1, total ash 2.3 ± 0.1, crude fat 16.3 ± 0.2, and crude protein 5.9 ± 0.5[14]. Many studies indicate that algal preparations have more protein content (Vieira *et.al*, 2020; Canelli *et.al., 2020*).

CONCLUSION

This study explored the use of *Chlorella vulgaris*, a microalgae strain, in nugget production, comparing it to traditional chicken nuggets and a vegetarian control sample. The microalgae culture showed optimal growth on the 15th day. DNA sequencing and blast analysis confirmed the identity of the cultured species. Sensory evaluationby a panel of individuals highlighted the acceptability and attributes of the microalgae nuggets, signalling their potential appeal in the consumer market. Proximate composition analysis revealed that *Chlorella vulgaris* nuggets had higher protein and lower fat content compared to chicken nuggets and the vegetarian control, showcasing their potential as a sustainable, nutritious protein source for eco-conscious and health-conscious consumers.

Looking ahead, the future holds promising perspectives for further research and development in this domain. In-depth exploration of scaling up microalgae-based nugget production, optimization of cultivation methods, and addressing sensory attributes for broader consumer acceptance will be pivotal. As sustainability and alternative protein sources continue to gain traction, *Chlorella vulgaris*- based nuggets could play a significant role in meeting the demands of a changing food industry and contributing to a more sustainable and diverse food ecosystem.

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