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Proximal, mineral, functional and bioactive attributes of the ectomycorrhizal edible mushroom *Clavaria versatilis*

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Abstract

The edible and ectomycorrhizal mushroom, *Clavaria versatilis*, is ubiquitous in Southwest India. It is an edible mushroom in southwest India. It is also ectomycorrhizal in commercial palms, *Areca catechu* and *Cocos nucifera*. It has proximal qualities, minerals, functional attributes and bioactive components. It possesses a high protein, fiber, carbohydrates and calorific value suitable for human diet. In this study, pressure cooking *Clavaria versatilis* with a low amount of water did not severely alter the contents of protein, total lipids and carbohydrates. It has sufficient magnesium content and the Ca/P ratio was desirable (>1) to prevent calcium loss and restoration. *Clavaria versatilis* possesses good water-absorption, oil-absorption and emulsion properties, which facilitate the preparation of functional foods. It is also known for pigments (carotenoids, β-carotene and lycopene), which are antioxidants and have health-promoting functions. Gas chromatography-mass spectrometry (GC-MS) showed a greater number of bioactive compounds in cooked samples compared to uncooked samples. Among the top five compounds, myristic and palmitic acids were common in uncooked and cooked samples. As *C. versatilis* is ectomycorrhizal in commercial palms (*Areca* and *Cocos*), it serves as a nutraceutical mushroom, warranting consideration for future benefits.

Keywords – Conservation – Mutualistic association – Nutritional potential– Pigments – Plantation crops – Southwest India

Introduction

The genus *Clavaria* is characterized by profusely branched basidiomes with a wide range of shades (bright yellow, orange, purple, red and white) and smooth-walled to warted echinulate to striated basidiospores. The branches of basidiome are divided into a slender base (stipe), distinct branches and apices (Exeter et al. 2006). This genus represents one of the largest groups in the family Gomphales (Kirk et al. 2008). There are reports of up to 18 species of *Clavaria* and allied species on many substrates (soil, humus, leaf litter, twigs, wood and roots) in the Western Ghats of India (Maharashtra, Karnataka and Kerala) (Table 1). Among these, several *Clavaria* spp. are edible and ectomycorrhizal with tree species.

The nutritional and bioactive potential of *Clavaria* spp. has been studied by many investigators. Sharma & Gautam (2017) evaluated the proximal, mineral, fatty acids, amino acids and bioactive profiles of 12 species of *Clavaria* found in the Northwestern Himalayas. *Ramaria*

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aurea consists of noteworthy amounts of proteins, fibre, minerals, carbohydrates and amino acids (Rai & Acharya 2012). *Ramaria botrytis* comprises unsaturated fatty acids and many bioactive components (e.g., ascorbic acid, β-carotene, flavonoids lycopene and tocopherol) (Barros et al. 2008, Sharma & Gautam 2017). *Ramaria subalpina* showed substantial antioxidant potential due to the presence of many bioactive compounds (Acharya et al. 2017). Fifty-three edible and 40 ectomycorrhizal species have been reported in Mexico, with 16 species possessing dual traits of edibility and ectomycorrhizal association (González-Ávila et al. 2013).

Table 1. *Clavaria*, *Ramaria* and allied species reported from the Western Ghats (* mycorrhizal; ** mycorrhizal and edible).

	Location	Substrate	Reference
***Clavaria versatilis* (Quél.) Sacc. & Trotter (Syn. *Ramaria versatilis* Quél.)	Mangalore University Campus (Karnataka)	Soil and roots of *Cocos nucifera*	Greeshma et al. (2016), Dattaraj et al. (2020)
	Kodandooru (Karnataka)	Soil and roots of *Areca catechu*	Present study
	Chandhakkunnu (Kerala)	Soil and humus	Mohanan (2011)
<u>*Phaeoclavulina eumorpha*</u> (P. Karast,) Giachini (Syn. *Ramaria eumorpha* (P. Karst.) Corner	Chandhakkunnu (Kerala)	Soil and humus	Mohanan (2011)
**Phaeoclavulina ipopelii* (lév.) Ocereem (Syn. *Ramaria zippelii* (Lév.) Corner	Mahabaleshwar (Maharashtra)	Soil	Senthilarasu (2013)
***Ramaria apiculata* (Fr.) Donk	Radhanagari (Maharashtra)	Soil	Thite et al. (1976), Patil & Thite (1977)
	Ammayambalam (Kerala)	Soil and humus	Mohanan (2011)
***Ramaria botrytis* (Pers.) Bourdot	Panhala (Maharashtra)	Humus-rich soil	Patil et al. (2016-2017)
	Shimoga (Karnataka)	Soil	Swapna et al. (2008)
Ramaria divaricata (Peck) Corner	Thusharagiri (Kerala)	Soil	Krishnapriya (2023)
***Ramaria flava* (Schaeff.) Quél.	Soil (Karnataka)	Soil	Karun & Sridhar (2016)
	Chandhakkunnu (Kerala)	Soil and humus	Mohanan (2011)
***Ramaria formosa* (Pers.) Quél.	Dandeli (Karnataka)	Soil	Ramesh & Pattar (2010)
	Chandhakkunnu (Kerala)	Soil and humus	Mohanan (2011)
Ramaria fragillima (Sacc. & P. Syd.) Corner	Vanaparvam & Thusharagiri (Kerala)	Soil	Krishnapriya (2023)
Ramaria gelatinosa Holmsk.	Mukkli (Kerala)	Wood	Krishnapriya (2023)
***Ramaria gracilis* (Pers.) Quél.	Soil (Karnataka)	Soil	Karun & Sridhar (2016)
	Peechi (Kerala)	Soil and humus	Mohanan (2011)
	Alaram (Kerala)	Twig	Krishnapriya (2023)
Ramaria grandis (Peck) Corner	Calicut University (Kerala)	Soil	Krishnapriya (2023)
***Ramaria pallida* (Schaeff.) Ricken	Konaje (Karnataka)	Soil	Karun & Sridhar (2014), Pavithra et al. (2016), Dattaraj et al. (2020)
	Sidhanpocket (Kerala)	Humus-rich soil	Mohanan (2011)
Ramaria pusilla Corner	Peruvannamuzhi (Kerala)	Soil	Krishnapriya (2023)
***Ramaria stricta* (Pers.) Quél.	Koodlu Theertha (Karnataka)	Soil	Prakash & Colney (2019)
	Alaram (Kerala)	Wood	Krishnapriya (2023)
Ramaria subaurantiaca Corner	Radhanagari (Maharashtra)	Soil	Thite et al. (1976), Patil & Thite (1977), Patil (1978)
	Palode (Kerala)	Soil and leaf litter	Krishnapriya (2023)
Ramaria subsigmoidea (Sacc. & P. Syd.) Corner	Vanaparvam (Kerala)	Soil	Krishnapriya (2023)
Ramaria suecica (Fr.) Donk	Mukkali (Kerala)	Wood	Krishnapriya (2023)

Sporocarps of *C. versatilis* occurring in the basins of coconut palms in scrub jungles were assessed for biochemical and bioactive potential in Dattaraj et al. (2020). Qualitative assays of uncooked samples revealed the existence of alkaloids, coumarins, flavonoids, saponins and terpenoids, whereas the cooked samples possessed alkaloids, cardiac glycosides, coumarins, saponins and terpenoids. Significantly higher measures of total phenolics and vitamin C were found in uncooked samples compared to cooked samples. The other antioxidant activities (DPPH radical-scavenging, ferrous ion-chelation and total antioxidant activities) were significantly high in uncooked samples.

Clavaria versatilis is one of the edible wild mushrooms in Nepal in the Western Himalayas (Christensen et al. 2008), hence this study aims at assessing the proximal, mineral, functional and bioactive properties of *Clavaria versatilis* found in the scrub jungles in the coastal region of south-western India. During mushroom expeditions in the scrub jungles of Mangalore University Campus (June-July 2021), we found an edible mushroom, *Clavaria versatilis* in ectomycorrhizal association with the roots in the soil around the basins of *Cocos nucifera* (coconut) palms during the southwest monsoon with a relative abundance between 0.6 and 1.7% (Greeshma et al. 2016, Dattaraj et al. 2020). This species was also found as ectomycorrhizal with palms of *Areca catechu* in a mixed plantation in the Kodandooru region of Manila Village, Dakshina Kannada, Karnataka, during the southwest monsoon season (June-July 2021).

Materials & Methods

The collected samples of *Clavaria versatilis* are shown in Fig. 1 and a schematic flow diagram to study the nutritional and functional properties is shown in Fig. 2.

Fig. 1 – *Clavaria versatilis* collected from the basins of coconut trees (*Cocos nucifeera*) (scale: cm).

Samples and process

Basidiocarps of *Clavaria versatilis* (Quél.) Sacc. & Trotter (Syn. *Ramaria versatilis* Quél.) were sampled from three locations in the basins of coconut trees in the scrub jungle of Mangalore University Campus (12°48’N, 74°55’E; altitude, 104–112 m asl). The collected samples were separately cleaned in water to remove debris and blotted with tissue. Each sample was divided into two groups: the first group served as the control (uncooked sample), while the second group was pressure-cooked with a small amount of water. The uncooked and cooked groups of samples were

dried (55±2°C) in an oven until reaching constant weight. Later, they were pulverized by a hand grinder to obtain fine flour. The samples were labelled and preserved in airtight bottles for one or two days for analysis.

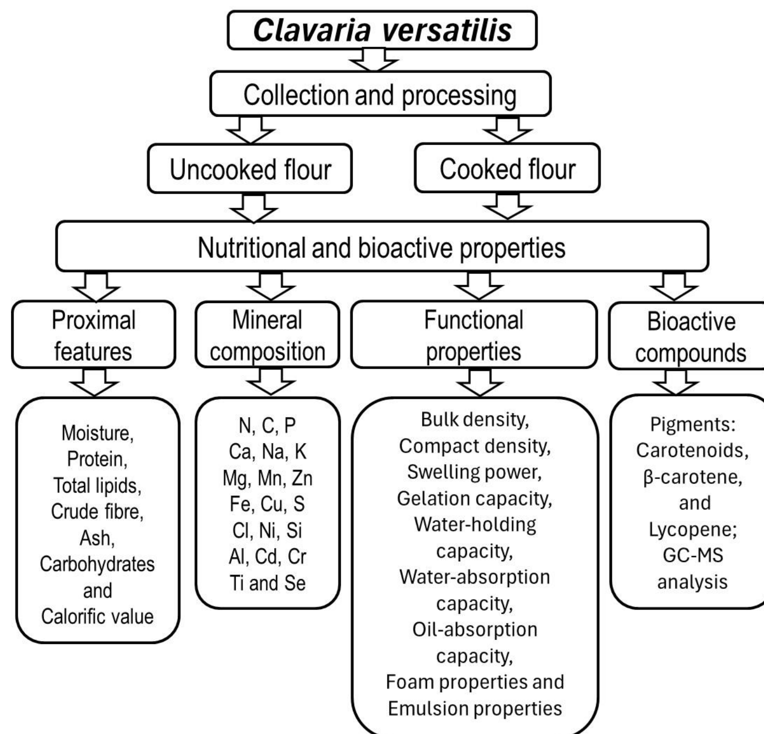

Fig. 2 – Schematic presentation of the analysis of *Clavaria versatilis*.

Proximal analysis

Moisture, protein, total lipids, fiber, ash, carbohydrates and energy of mushroom samples were determined for three samples collected independently.

To determine the moisture content, dried mushrooms (0.5 g) were taken on a pre-weighed dried Petri plate. It was then dried in an oven (100 °C, up to 6 hr) followed by cooling in a desiccator. The final weight of the Petri plate with the dried sample was noted. The moisture content was calculated in percent based on the variation between the initial weight and the final weight.

To estimate protein content, mushroom flour (500 mg) was ground using a pestle and mortar with phosphate buffer (5−10 ml). After centrifuging (5,000 rpm, 5 min), the supernatant was considered for protein estimation (Lowry et al. 1951). The working standard was prepared using bovine serum albumin. Different amount of supernatant (0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml) was made up to 1.0 ml (except the last tube) with distilled water in test tubes (the first tube with plain distilled water served as a blank). Alkaline copper solution was added to each tube (5 ml), vortexed and kept aside (10 min). Then Folio-Ciocalteu's reagent was added (0.5 ml), mixed and incubated (30 min) at laboratory temperature (27±2 °C). The absorbance was read at 660 nm and a standard graph was plotted for the calculation of protein in the sample.

The extraction of total lipids was carried out using the cold extraction method by Bligh & Dyer (1959). Mushroom flour (1 g) was homogenized with a solvent mixture (chloroform methanol, 2:1 v/v) and preserved overnight. The content was transferred to a separatory funnel containing double-distilled water (10 ml), mixed and allowed to separate. The lipid containing the lower chloroform layer was transferred into a pre-weighed beaker and allowed to evaporate to dryness at room temperature (27±2 °C) and the quantity of lipid extracted was determined gravimetrically.

To assess crude fibre, mushroom flour was added to a beaker (1 g) with sulphuric acid (0.255 N, 200 ml) and the mixture was boiled (1 hr) with continuous stirring while maintaining a constant volume with the addition of distilled water (AOAC 1999). The material was transferred to a beaker and NaOH was added (0.313 N, 200 ml) and boiled (1 hr) with constant stirring. The contents were filtered through a multilayered muslin cloth and repeatedly washed with hot distilled water. Thereafter, the filtrate was washed with alcohol and the filtered material was collected in a pre-weighed crucible. It is dried in an oven (80 °C) and transferred to a furnace (600 °C) until the material transformed into ash. The difference in weight between the empty crucible and the crucible with ash was reported as the fiber content.

The ash content of mushroom flour was assessed in percentage (AOAC 1999). The mushroom flour (1 g) was transferred into a pre-weighed porcelain crucible and allowed to dry in the oven (100 °C, 6−8 hr). Later, the crucible was moved to a furnace to attain a constant weight (550 °C, 8 hr) and the ash content was calculated gravimetrically.

The carbohydrate content of the flour was calculated based on the quantities of protein, lipid, fiber and ash (Müller & Tubin 1980).

Carbohydrate (%) = 100 − (crude protein + crude lipid + crude fiber + ash)

The calorific value of mushroom flour was estimated based on the amount of protein, lipids and carbohydrates (Ekanayake et al. 1999).

Calorific value (kJ/100 g) = (Protein × 4) + (Total lipids × 9) + (Carbohydrates × 4)

Mineral assay

The mineral composition of mushroom flour was evaluated based on Ramamurthy & Kannan (2009). The flour (free from moisture) was assessed by the field-emission scanning electron microscope-energy dispersive spectrometer analysis (SEM-EDS) (FESEM Carl Zeiss, Oxford Instruments, USA) at 15 kV. The SEM images and the EDS spectrum of the samples rely on the shape, shell and size of mushroom particles to calculate the mineral contents. The Na/K as well as Ca/P ratios of the samples were calculated.

Functional qualities

Bulk density was determined by transferring mushroom flour (20 g) to a measuring jar. The volume occupied by the sample was noted to calculate the ratio in g/ml (Singh et al. 2005).

To determine compact density, mushroom flour (10 g) was transferred into a measuring cylinder. The cylinder with the sample was vortexed (3 min) to obtain a constant volume, which was measured.

To find swelling power, mushroom flour (0.5 g) was taken in a pre-weighed centrifuge tube and reweighed. Distilled water (10 ml) was added and the flour was allowed to swell in a boiling water bath (30 min), cooled and centrifuged (5,000 rpm, 10 min). The decanted supernatant was transferred to a test tube and the weight of the centrifuge tubes was taken with a swollen sample to calculate the swelling power per gram.

To assess the gelation capacity, mushroom flour was dispensed in 10 test tubes (0.2 g to 2 g) and distilled water was added to each tube (10 ml). The tubes were kept in a hot water bath (80 °C, 30 min). Tubes were cooled to room temperature and re-incubated (4 °C, 1 hr). Each tube was inverted to observe the stability of the sample without slipping. The lowest gelation concentration was noted to ensure that the concentration in the gel remained constant.

To find out the water-holding capacity, 5 g of mushroom flour was added to test tubes and distilled water (10 ml) was loaded through the sides of the tubes (Bauchat 1977). The tubes were incubated at room temperature (26±2 °C, 24 hr). Later, the wet powder in the tubes was transferred into pre-weighed petri plates. The weight of the petri plates with the powder was noted. The petri plate with the wet sample was placed in a hot air oven for drying (105 °C, 6 hr). After drying, the weight of the dried sample on the petri plate was determined. The difference between the weight of the wet sample and the dried sample was reported as the water content that is considered as the water-holding capacity.

To assess the water-absorption capacity, mushroom flour (1 g) was loaded into a graduated centrifuge tube with distilled water (10 ml) and incubated for 30 min (27±2 °C). After being centrifuged (5,000 rpm), the height of the supernatant was noted to express the water-absorption in ml per gram.

To assess the oil-absorption capacity, mushroom flour (0.5 g) was added to a graduated centrifuge tube with edible oil (household coconut oil, 5 ml) and allowed to stand (26±2 °C; 30 min) (Baucvjat 1977). Later, the contents of the tubes were centrifuged (5,000 rpm; 30 min). The supernatant volume was measured as the quantity of oil absorbed in ml per gram of flour.

To determine foam capacity, mushroom flour (2 g) was mixed with distilled water (100 ml), then loaded into a measuring jar and the total volume was noted. The entire content was added to a blender and mixed vigorously (2 min) (Coffman & Garcia 1977). The mixture was transferred into a measuring jar to note the total volume to determine the foam capacity.

To assess foam stability, mushroom flour (2 g) was mixed with distilled water (100 ml) and transferred into a measuring jar. It was blended vigorously (2 min) to generate the foam (Coffman & Garcia 1977). The volume of foam in the measuring jar was noted and kept at laboratory temperature (27 ± 2 °C, 8 hr) and the final stable foam volume was noted to find the foam stability.

To find out the emulsion capacity, mushroom flour (0.5 g) was suspended in distilled water (5 ml) and the solution height in the cylinder was noted (Neto et al. 2001). After homogenization with refined coconut oil (2.5 ml), it was centrifuged (3,000 rpm, 5 min). The emulsified layer height was measured.

To assess emulsion stability, the emulsion was subjected to high temperatures in water bath (85 °C, 15 min) (Neto et al. 2001). Later, it was left at room temperature (26±2 °C, 30 min) to cool. The emulsified layer height was recorded to determine the emulsion's stability.

Pigment analysis

The cooked and uncooked mushroom flour were extracted (90% acetone) for the analysis of carotenoids by determining the absorbance (480, 510 and 750 nm) (Nagata & Yamashita 1992).

Carotenoids (mg/100 mg) = $7.6 (A_{480} - A_{750}) - 1.49 (A_{510} - A_{750})$

A ratio of 4:6 acetone and hexane mixture was used for the analysis of the amounts of β-carotene and lycopene contents, respectively (Nagata & Yamashita 1992). The β-carotene and lycopene were absorbed at wave lengths of 453, 505 and 663 nm. The extract was centrifuged, the supernatant was taken to observe the absorbance at the respective wavelengths and to calculate the quantity of pigments.

β-carotene (mg/100 mg) = $(0.216 \times A_{663}) - (0.304 \times A_{505}) + (0.452 \times A_{453})$

Lycopene (mg/100 mg) = $(0.0458 \times A_{663}) + (0.372 \times A_{505}) - (0.0806 \times A_{453})$

Analysis of bioactive components

Bioactive compounds of *C. versatilis* ethyl acetate extracts were examined using GC-MS (Scion 436-GC Bruker model coupled with a triple quadrupole mass spectrophotometer). The operation conditions included the fused silica capillary column BR-5MS. Helium gas was employed as a carrier at a constant flow (1 ml/min) and an injection (2 µl) was used (split ratio, 10:1). The oven temperature of the column was set to 110 ºC hold for 3.5 min, up to 200 ºC at the rate of 10 ºC/min-no hold, up to 280 ºC at the rate of 5 ºC/min-9 min hold. The injector temperature was 280 ºC and the inlet source temperature was 290 and 250 ºC, respectively. The mass spectrometer was operated in the positive electron ionization (EI) mode with 70 eV ionization energy. The solvent delay was 0–3.5 min and the total GC-MS running period was 39 min. The relative percent of components was calculated based on the average peak area of the total areas.

Data analysis

The *t*-test (Statistica, Version # 8, 2008) was used to determine the difference in proximal properties, functional attributes and pigments of uncooked and cooked mushroom flours.

Results and Discussion

Proximal value

The moisture content was higher in cooked samples compared to uncooked samples ($p<0.05$) (Table 2). The protein content decreased with cooking ($p<0.05$). The total lipid content was improved in cooked samples ($p<0.05$). Cooking raised the fiber content ($p<0.001$). Ash content was lower in cooked than uncooked samples ($p<0.05$). Carbohydrate contents were also higher in uncooked samples compared to cooked samples ($p<0.05$). The calorific value was higher in cooked than uncooked samples without a significant difference ($p>0.05$).

The protein content of *C. versatilis* was lower compared to six *Ramaria* spp. studied by Sharma & Goutam (2017) (7.5 % vs. 10.8–21.7 %). The total lipids of uncooked samples were comparable to those of *R. flava*, *R. flavescens* and *R. rubripermanens*, while cooked samples contain *R. aurea, R. botrytis* and *R. stricta*. The crude fiber was extremely high in *C. versatilis* compared to other six *Ramaria* spp. (12.7–13.9 vs. 0.3–14.3). The ash content in *C. versatilis* was extremely high compared to the other six *Ramaria* spp. (3.2–4.1 % vs. 0.2–1.3 %), which is reflected in higher mineral content. Similar to fiber and ash in *C. versatilis*, carbohydrate content was also higher than in the six *Ramaria* spp (74–75 % vs. 41–50 %).

Table 2. Proximal composition of *Clavaria versatilis* on a dry mass basis (n=3, mean±SD; *t*-test: *, <0.05; **, <0.001).

	Uncooked	Cooked
Moisture (%)	1.03±0.15	1.46±0.05*
Proteins (%)	17.77±0.25*	16.43±0.45
Total lipids (%)	0.43±0.15	1.43±0.21*
Crude fiber (%)	12.73±0.24	13.91±0.35**
Ash (%)	4.06±0.61*	3.20±0.39
Carbohydrates (%)	65.92±0.56	66.18±0.24
Calorific value (kJ/100 g)	336.97±1.90	343.37±2.86

The protein content of *C. versatilis* was closer to that of green gram (*Vigna radiata*) (16–18 % vs. 16–17 %) (Oghbaei & Prakash 2017). However, the low content of total lipids and high levels of fiber, carbohydrates and energy could be suitable for human diet. The high fibre content is beneficial to human nutrition as it facilitates the improvement of digestibility (by trapping less proteins and carbohydrates), drops blood cholesterol and fights bowel as well as colon cancers (Bologun & Fetuga 1986, Anderson et al. 1995, Salvin et al. 1997). The high fibre content leads to a delay in the conversion of starch into simple sugars, which helps control diabetes (Ogbonnaya & Chinedum 2013). Similarly, high carbohydrate content is the major source of energy, which is capable of preventing intestinal cancers as well as type II diabetes (Venn & Mann 2004). The energy value did not alter much during cooking owing to a meagre loss of proteins, lipids and carbohydrates. The nutritional quality could be retained fairly well by adapting suitable thermal processing.

Mineral composition

The mineral content of *C. versatilis* decreased after cooking, which is also reflected in the decreased ash content (Table 3). The carbon content was higher than other minerals, followed by nitrogen, sodium, calcium and potassium. Seventeen minerals were higher in uncooked samples compared to cooked samples. Among the minerals found in *C. versatilis* based on SEM-EDS, they were devoid of nickel, while cadmium and chromium were below detectable levels, whereas aluminium content was fairly low.

Sodium is a dominant mineral, followed by calcium, phosphorus, potassium and iron. The Na/K ratio and the Ca/P ratio were also higher in the uncooked samples. Although the Na/K ratio is not advantageous (the requirement is <1), the Ca/P ratio (>1) can be useful to prevent calcium loss

in urine and its restoration in bones (Yusuf et al. 2007). Besides, calcium is involved in the prevention of rickets as well as osteoporosis (Agunbiade et al. 2015), while magnesium is active against coronary diseases and stroke (Rosque-Esteban et al. 2018).

Functional properties

Among the 11 functional parameters studied, *C. versatilis* lacked foam capacity as well as foam stability (Table 4). Bulk density, as well as water- and oil-absorption capacities were higher in uncooked compared to cooked samples ($p<0.05$). Compact density, swelling power, least gelation capacity, emulsion activity and emulsion stability were higher in cooked samples compared to uncooked samples ($p<0.05$).

Both uncooked and cooked samples of *C. versatilis* possess good swelling power, however, the lowest gelation concentration increased with cooking, thus requiring a suitable thermal process that decreases the lowest gelation concentration. Water- as well as oil-absorption capacities were fairly high, although they decreased with cooking. These qualities are important to formulate food with good texture as well as mouthfeel (e.g., doughnuts and soups) (Alobo 2003). Owing to the good emulsion activity and emulsion stability of *C. versatilis*, it will be helpful to prepare value-added beverages. As *C. versatilis* is devoid of foam properties, it is not suitable to prepare foods such as desserts and cakes (Niveditha & Sridhar 2017).

Table 3. Mineral composition (atomic%) of *Clavaria versatilis* on a dry mass basis (BDL, below detectable limit; NP, not present).

Mineral	Uncooked	Cooked
N	7.690	6.582
C	29.98	28.25
P	2.582	2.265
Ca	3.952	3.785
Na	4.218	3.820
K	2.185	1.952
Mg	1.482	1.325
Mn	0.078	0.038
Zn	0.836	0.654
Fe	1.983	1.922
Cu	1.038	1.012
S	0.144	0.135
Cl	0.012	0.008
Ni	NP	NP
Si	0.857	0.310
Al	0.079	0.062
Cd	BDL	BDL
Cr	BDL	BDL
Ti	0.0022	0.0018
Se	0.0182	0.0148
Na/K ratio	1.93	1.96
Ca/P ratio	1.53	1.67

Pigments

Among the three pigments assessed in *C. versatilis,* carotenoids were higher in uncooked compared to cooked samples ($p<0.001$) (Fig. 3). β-carotene was higher in cooked than uncooked samples ($p<0.001$). Lycopene content was higher than in the cooked samples ($p<0.001$). These bioactive pigments have been reported in many species of *Ramaria* by Sharma & Gautam (2017). Carotenoids are not synthesized de novo in humans, thus needs to be acquired through diet (Rasmus & Kazłowska 2023). They are effective antioxidants involved in the reduction of inflammation. β-carotene serves as a potent antioxidant and prevents eye diseases. Lycopene is known to improve cellular antioxidant defence and is beneficial for bone health and aid in cancer treatment, thus, it serves as a potent sunscreen.

Table 4. Functional properties of *Clavaria versatilis* (n=3±SD; *t*-test: *, 0.05; **, 0.01; ***, 0.001).

Properties	Uncooked	Cooked
Bulk density (g/ml)	0.83±0.04***	0.56±0.04
Compact density (g/ml)	0.43±0.02	0.54±0.01**
Swelling power (g/g)	2.12±0.03	3.24±0.14**
Least gelation capacity (%)	17.57±0.45	19.36±0.55**
Water-holding capacity (%)	18.25±0.25	19.03±0.03*
Water-absorption capacity (ml/g)	5.73±0.25**	3.37±0.54
Oil-absorption capacity (%)	3.26±0.24*	3.05±0.15
Foam capacity (%)	0	0
Foam stability (%)	0	0
Emulsion activity (%)	40.20±0.40	42.83±0.25***
Emulsion stability (%)	43.10±0.003	44.77±0.56**

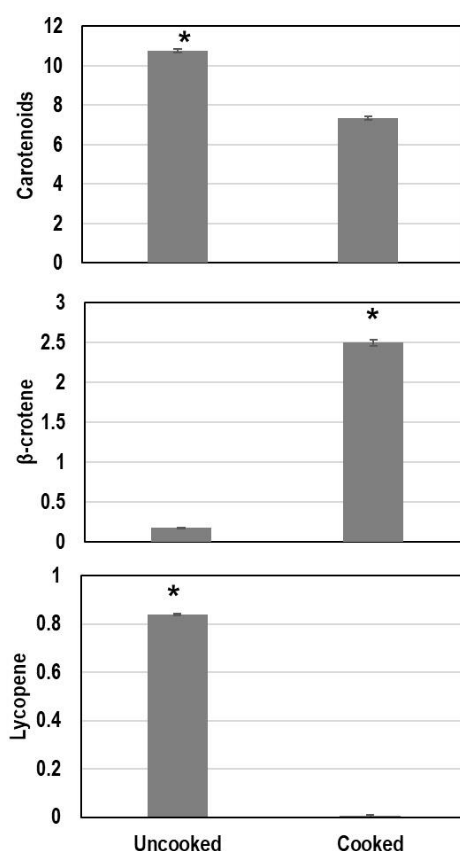

Fig. 3. Carotenoids, β-carotene and lycopene contents in uncooked and cooked *Clavaria versatilis* (n=3±SD; *t*-test: *, p<0.001).

Bioactive components

The GCMS analysis of ethyl acetate of uncooked and cooked *C. versatilis* showed 25 and 29 components, respectively (Fig. 4). Many compounds were common in uncooked and cooked samples. The major component in the uncooked sample was n-hexadecanoic acid (palmitic acid), followed by 1-Hexadecanol (cetyl alcohol) and tetradecanoic acid (myristic acid) (Table 5). The major components in the cooked sample were dodecanoic acid, 1,2,3-propanetriyl ester (trilaurin), followed by n-hexadecanoic; rac-glycerol-1,3-dilaurate; 9-Octadecenoic acid, 1,2,3-propanetriyl ester; tetradecanoic acid (myristic acid); Glycerol tricaprylate; glycerol tricaprylate and octadecanoic acid (Table 6). Palmitic acid is one of the components of dairy products, meat and oils. It is used in soaps, cosmetics, as a mouthfeel and in several food. Cetyl alcohol serves as an emulsifier and possesses antimicrobial activity, hence, it is used in wound dressings and skin creams (Smolinske 1992). Myristic acid is known to increase low-density lipoprotein (LDL) in the blood (Schwingshackl et al. 2018), while trilaurin is used in sunscreen products.

Conclusions and outlook

Clavaria versatilis was found in different parts of India and possess nutritional, medicinal, bioactive and mycorrhizal attributes. In southwest India, it has been reported from Maharashtra, Karnataka and Kerala. This species is known from different locations of scrub jungles in the southwest of Karnataka and mycorrhizal with commercial palms (*Areca catechu* and *Cocos nucifera*). A few studies have been carried out on the importance of *C. versatilis*, and it has appreciable proximal qualities, minerals, bioactive potential and functional attributes. It possesses protein equivalent to green gram and also possesses high fiber, carbohydrates and calorific value suitable for human diet. Conventional pressure cooking with a low amount of water also do not drastically alter the contents of protein, total lipids and carbohydrates.

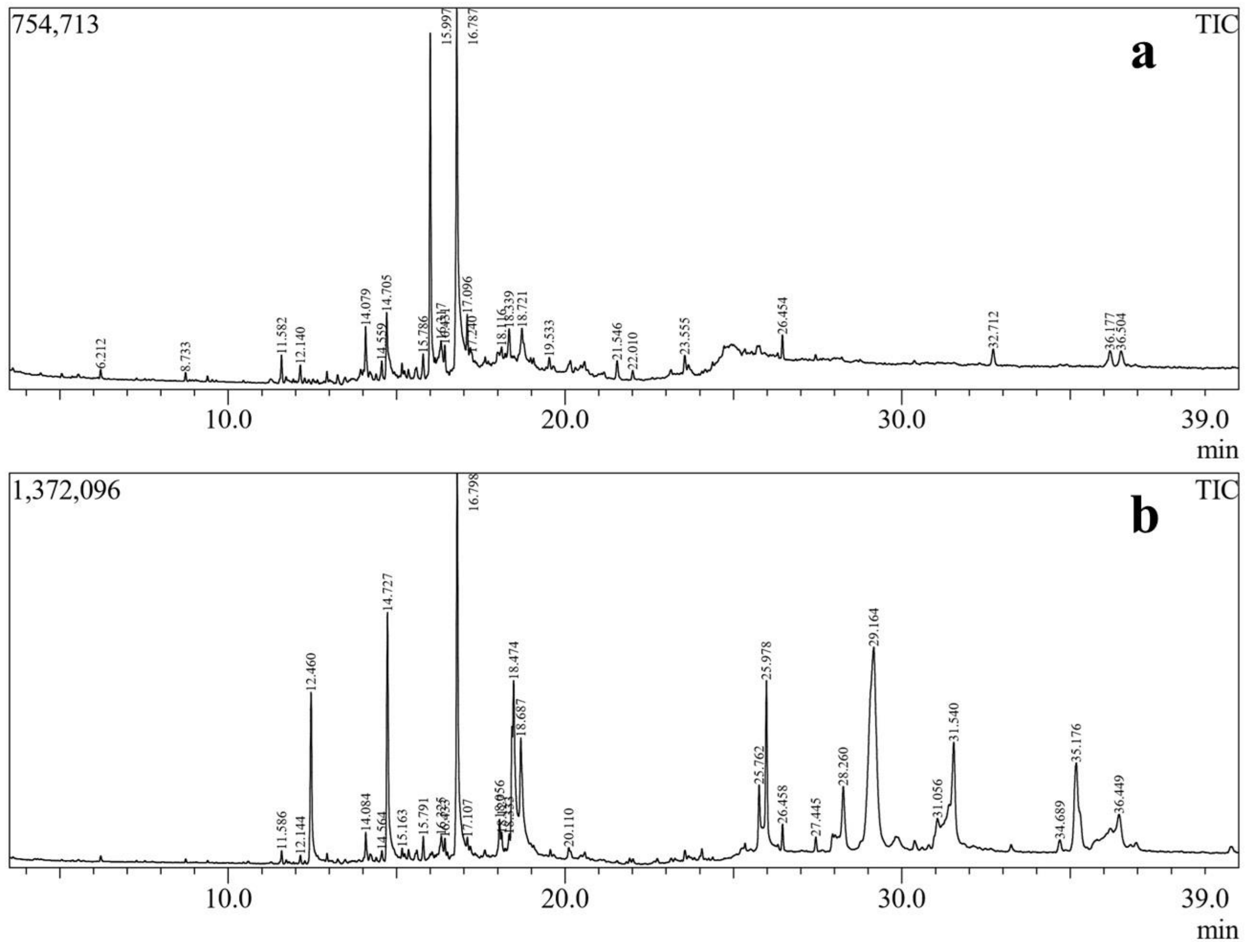

Fig. 4. Chromatogram of GC-MS analysis of uncooked (a) and cooked (b) *Clavaria versatilis*.

Among the minerals, sodium was dominant, followed by calcium, phosphorus, potassium and iron. The Ca/P ratio was desirable (>1) to combat calcium loss in urine, its restoration in bones and the prevention of osteoporosis. It has sufficient magnesium content to prevent coronary diseases and strokes. It possesses good water-absorption, oil-absorption and emulsion properties, which help in the preparation of foods with better texture and mouthfeel. It also possesses good quantities of pigments (carotenoids, β-carotene and lycopene), which serve in human health, particularly as antioxidants, skin care and cancer treatment. Among the top five compounds obtained from GC-MS, myristic and palmitic acids were common. Since *C. versatilis* is ectomycorrhizal in palms like *Areca* and *Cocos*, it caters to the nutritional demands of commercial palms. It also serves as a nutraceutical source for humans during the southwest monsoon and demands conservation and cultivation for future benefits.

Table 5. Bioactive constituents in the ethyl acetate extract of uncooked *Clavaria versatilis* by GC-MS.

Retention time (min)	Active principle	Peak area (%)	Formula
6.212	Ethyl 3-acetoxybutyrate	0.48	$C_{16}H_{34}$
8.733	Nonane, 1-iodo-	0.43	$C_9H_{19}I$
11.582	Dodecane, 4,6-dimethyl-	1.43	$C_{14}H_{30}$
12.14	Dodecane, 4,6-dimethyl-	1.11	$C_{14}H_{30}$
14.079	Triacontane, 1-iodo-	2.78	$C_{30}H_{61}I$
14.559	3-Ethyl-2,6,10-trimethylundecane	1.09	$C_{16}H_{34}$
14.705	Tetradecanoic acid (Myristic acid)	6.28	$C_{14}H_{28}O_2$
15.786	Phthalic acid, hept-2-yl isobutyl ester	1.68	$C_{19}H_{28}O_4$
15.997	1-Hexadecanol (Cetyl alcohol)	20.95	$C_{16}H_{34}O$
16.317	Dotriacontane	1.65	$C_{32}H_{66}$
16.431	Hexadecanoic acid, methyl ester	0.97	$C_{17}H_{34}O_2$
16.787	n-hexadecanoic acid (Palmitic acid)	37.94	$C_{16}H_{32}O_2$
17.096	Hexadecanoic acid, ethyl ester	3.3	$C_{18}H_{36}O_2$
17.24	Octacosyl acetate	1.59	$C_{30}H_{60}O_2$
18.116	1-Methoxyoctacosane	1.88	?
18.339	2-Methylhexacosane	2.22	$C_{27}H_{56}$
18.721	Triarachine	3.27	$C_{63}H_{122}O_6$
19.533	2H-Pyran-2-one, tetrahydro-6-tridecyl-	0.76	$C_{18}H_{34}O_2$
21.546	Methyl tetratriacontyl ether	1.4	$C_{35}H_{72}O$
22.01	Pentadecanal-	0.61	$C_{15}H_{30}O$
23.555	Pentadecanal-Phthalic acid, di(6-methylhept-2-yl) ester	1.24	$C_{24}H_{38}O4$
26.454	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetraco	1.36	$C_{30}H_{54}O_2$
32.712	E,Z-2,15-Octadecadien-1-ol acetate	1.51	$C_{20}H_{36}O_2$
36.177	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomet	2.02	$C_{15}H_{25}I$
36.504	Hexadecanoic acid, octadecyl ester	2.03	$C_{34}H_{68}O_2$

Table 6. Bioactive constituents in the ethyl acetate extract of cooked *Clavaria versatilis* by GC-MS.

Retention time (min)	Active principle	Peak area (%)	Formula
11.586	3-Ethyl-2,6,10-trimethylundecane	0.25	$C_{16}H_{34}$
12.144	3-Ethyl-2,6,10-trimethylundecane	0.19	$C_{16}H_{34}$
12.46	Dodecanoic acid (Lauric acid)	4.85	$CH_3(CH_2)_{10}COOH$
14.084	Eicosane	0.58	$C_{20}H_{42}$
14.564	Triacontane, 1-iodo-	0.3	$C_{30}H_{61}I$
14.727	Tetradecanoic acid (Myristic acid)	7.67	$C_{14}H_{28}O_2$
15.163	Heneicosane	0.15	$C_{21}H_{44}$
15.791	Phthalic acid, hept-2-yl isobutyl ester	0.52	$C_{19}H_{28}O_4$
16.325	10-Methylnonadecane	0.67	$C_{20}H_{42}$
16.433	Hexadecanoic acid, methyl ester	0.33	$C_17H_{34}O_2$
16.798	n-Hexadecanoic acid (Palmitic acid)	12.94	$C_{16}H_{32}O_2$
17.107	Eicosanoic acid, ethyl ester	0.18	$C_{22}H_{44}O_2$
18.056	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.83	$C_{19}H_{34}O_2$
18.127	9-Octadecenoic acid, methyl ester, (E)-	0.4	$C_{19}H_{34}O$
18.333	2-Methylhexacosane	0.4	$C_{72}H_{56}$
18.474	9-Octadecenoic acid, 1,2,3-propanetriyl ester,	9.38	$C_{57}H_{104}O_6$
18.687	Octadecanoic acid	5.33	$CH_3(CH_2)_{16}COOH$
20.11	Myristic acid glycidyl ester	0.47	?
25.762	1-Hydroxy-3-(octanoyloxy)propan-2-yl decan	2.12	$C_{21}H_{40}O_5$
25.978	Decanoic acid, 2-hydroxy-3-[(1-oxooctyl)oxy]p	5.41	$C_{21}H_{40}O_5$
26.458	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracos	0.6	$C_{30}H_{54}O_2$
27.445	Hexacontane	0.37	$C_{60}H_{122}$

Table 6. Continued.

Retention time (min)	Active principle	Peak area (%)	Formula
28.26	1-Decanoyl-3-dodecanoylglycerol	2.35	$C_{18}H_{38}NO_7P$
29.164	Dodecanoic acid, 1,2,3-propanetriyl ester	23.04	$C_{39}H_{74}O_6$
31.056	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethan	2.16	$C_{35}H_{68}O_5$
31.54	Rac-glycerol-1,3-dilaurate	9.82	$C_{27}H_{52}O_5$
34.689	Cholest-5-ene, 3-methoxy-, (3.beta.)-	0.55	$C_{28}H_{48}O$
35.176	Glycerol tricaprylate	6.81	$C_{27}H_{50}O_6$
36.449	1-Dodecanoyl-3-myristoylglycerol	1.32	$C_{41}H_{78}O_6$

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