Food Research 7 (Suppl. 4) : 37 - 45 (2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Effects of solid-state fermentation of seaweed (*Caulerpa racemosa*) on antioxidant assay and flavour profile

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Article history:
Received: 29 January 2023
Received in revised form: 17 June 2023
Accepted: 9 March 2023
Available Online: 11 November 2023

Keywords:
Caulerpa racemosa,
Antioxidant assay,
Flavour profile,
Sensory evaluation,
Solid-state fermentation,
Satiety

DOI:
https://doi.org/10.26656/fr.2017.7(S4).4

Abstract

The effects of solid-state fermentation (SSF) of *Caulerpa racemosa* on antioxidant properties (total phenolic content (TPC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferric-reducing antioxidant power (FRAP)) and flavour profile were investigated. Additionally, sensory acceptability (9-Point Hedonic Test) and satiety analysis (10-Scale Intention) of oat drinks incorporated with SSF of *C. racemosa* were also studied. Results of SSF *C. racemosa* demonstrated that significantly ($p<0.05$) higher content in TPC, DPPH, IC50 and FRAP (312.30±5.76 mg GAE/100 g, 25.96±0.17 mg AA/100 g, 46.22±0.31 mg/mL, 1106.72±21.82 mg TE/100 g and 206.80±4.76 mg GAE/100 g, respectively). Flavour profile analysis indicated the presence of antimicrobial volatile siloxane compounds; hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane. These -siloxane group compounds are known to have antioxidant, defoaming, and release coating properties. In addition, results obtained from the sensory evaluation showed that the addition of a different amount of SSF *C. racemosa* did not significantly ($p>0.05$) affect the acceptability of instant oat drinks. However, oat drinks incorporated with SSF *C. racemosa* significantly ($p<0.05$) increased in fullness from the satiety study. Therefore, value-added SSF *C. racemosa* can be incorporated into food products to increase its nutritional properties and fullness without affecting the taste.

1. Introduction

Seaweed is known as the sea creature with the highest levels of natural antioxidants (Michalak *et al*., 2022), antibacterial (Chakraborty *et al*., 2010) and antimicrobials, including polyunsaturated fatty acids, polysaccharides, phlorotannins, carotenoids and other phenolic compounds (Pérez *et al*., 2016). Additionally, seaweeds have minimal calories, high levels of vitamins, fibre, trace minerals, and a variety of secondary metabolites (Pulz and Gross, 2004). Seaweeds like *Caulerpa lentillifera, Caulerpa racemosa*, and *Solieria* sp. are harvested from the wild and *Kappaphycus* farms and sold alongside *Eucheuma* and *Kappaphycus* as seen in the markets of Sabah. *Eucheuma*, *Kappaphycus* and *Gracilaria* dominate the seaweed commodity in Malaysia (Phang *et al*., 2019). One of the economically valuable green algal seaweed products known as sea grapes is *Caulerpa racemosa* (Paul *et al*., 2019) and has a high mineral content (calcium and magnesium), high fibre, folic acid, proteins, vitamin B1, ascorbic acid, and vitamin A, and has a low-fat content (Matanjun *et al*., 2009). The seaweed used in this investigation is *C. racemosa* and solid-state fermentation (SSF) was employed to improve polyphenol bioavailability and lessen the unpleasant smell of this seaweed.

Cereals made from whole grains are regarded as an essential component of diet and nutrition because of their numerous health advantages (Bouchard *et al*., 2022). Oats (*Avena sativa*) are a good source of protein, β-glucan, unsaturated fatty acids, and the antioxidant-rich avenanthramides polyphenols (Li *et al*., 2022). Antioxidants known as avenanthramides were formerly thought to be phytoalexins that plants release in reaction to diseases like fungi (Perrelli *et al*., 2018). As part of a balanced diet, oats are transitioning from a nutritional staple for feed and food to a source of nutritious whole grains. Several health benefits have been accepted by the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) in the United States (FDA) (Smulders *et al*., 2018), which contain qualifying sources of soluble fibre that may lower the risk of heart disease via the intermediary connection of "blood

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

cholesterol" or "blood total- and LDL-cholesterol," and also in conformity with the American Dietary Guidelines.

The degree and duration of post-meal feelings of satiety are influenced not just by the caloric content but also by the composition of the food ingested. This is due to the dietary fibre content of oats. This dietary trait can increase satiety during meals while lowering food intake. In porridge/satiating drink's abilities, the combination of low energy density, high dietary fibre content, and a naturally intact structure makes it an excellent alternative for the creation of satiating meals and diets (Isaksson *et al*., 2012).

Previous studies have effectively documented the increment of antioxidant activity and phenolics content in the solid-state fermented product. The effects of SSF *Kappaphycus* spp. by using *Aspergillus oryzae* significantly increased the bioavailability of phenolic compounds, amino acids and volatiles (Norakma *et al*., 2022). Similar to the result, SSF *Saccharina japonica* with *Monascus purpureus* exhibited significant bio-functional properties (increased flavonoids and phenolics contents), has robust in vitro biopotential, DNA protection, and no toxicity to intestinal epithelial cells, the Caco-2 cells (Suraiya *et al*., 2018). In the SSF, peanut press cake powder significantly increased the enzymatic activity, thus enhancing the bioavailability and mineral content in enriched sweetened yoghurt cheese (Duhan *et al*., 2020). Moreover, compared to quinoa and wheat, SSF lupin fermented by the novel probiotics *Lb. reuteri* and *Lb. plantarum* K779 showed distinct antiproliferative effects (Ayyash *et al*., 2019). However, research has yet to be done on the investigation of solid-state fermented *C. racemosa* application. Therefore, the focus of this study was to examine the impact of SSF of *C. racemosa* on antioxidant assay and flavour profile. Effects addition of SSF *C. racemosa* in a food product, an instant oat drink, has also been studied for sensory evaluation and satiety.

2. Materials and methods

2.1 Materials

The instant oatmeal was purchased from Pepsico (Malaysia) Sdn. Bhd and was stored in a cool and dry place to avoid direct heat and sunlight until further analysis.

2.2 Sample preparation

For this study, *C. racemosa* seaweed was collected from Langkawi Island in Malaysia. *C. racemosa* and was soaked in a 1:2 ratio of water (g:mL) for 1 h after being washed with tap water. After being sliced and dried for 5 days at room temperature, the samples were ground and fermented using *Aspergillus oryzae* for 4 days at 30°C as used by Tan and Lee (2014) with some modification.

Aspergillus oryzae bacteria that was used for this study was obtained from the MARDI culture collection in Serdang, Selangor. For four days, the strain was grown on PDA plates at 30°C. Spores were obtained by using a hockey stick to evenly distribute 100 mL of sterile distilled water across four PDA plates holding a four-day-old culture. Following filtration using Whatman filter paper no 1, the suspended fungal cultures were used as an inoculum for solid-state fermentation. Before use, the inoculum was stored at 4°C for a month.

About 1 g of the freeze dried (Alpha 1-4 LDplus Freeze Dryer from Christ, Germany) sample was added into 50 mL methanol (50%) (HmbG Chemicals, Germany). This was followed by homogenizing (T25 digital Ultra Turrex® Homogenizer from IKA®, Germany) for about 4 mins. Before centrifuge (Tabletop Centrifuge 5420 from Kubota, Japan) for 15 mins at 3,500 rpm, the mixture was shaken thoroughly by using an orbital shaker (Innova 4080 Incubator Shaker from New Brunswick Scientific, USA) for 1 hr at 200 rpm. The samples were filtered using Whatman paper number 1 and stored at -20°C until further analysis (Chew *et al*. 2008).

2.2 Total phenolic content

Determination of TPC for both unfermented and fermented extracts in 50% methanol was carried out following the method used by Chew *et al*. (2008) with slight modification, TPC was determined using the Folin -Ciocalteu (FC) reagent (R&M Chemicals, UK) in triplicates. Sample extract (0.3 mL) was combined with 7.5% w/v Na_2CO_3 (Systerm, Malaysia), 1.5 mL of Folin Ciocalteu's phenol reagent, and 1.2 mL of sample extract. The reaction mixture was left to sit for 30 mins in the dark after being vortexed by using Vortex Mixer LM-3000 (Labmart, Malaysia). The absorbance of the reaction mixture was then measured at 765 nm (UV-VIS Spectrophotometer from GENESYS™ 20, Germany). Gallic acid equivalents (GAE)/100 g sample was used to compute TPC. The standard curve of gallic acid (Sigma, Japan) was made with the gallic acid concentration of 0-100 mg/L. $Y = 0.0145x \pm 0.0074$ ($R^2 = 0.9969$) was the calibration equation for the standard curve of gallic acid. The TPC value was calculated using the following equation:

$$\text{PC value} = \frac{\text{raw TPC value x dilution factor x volume of the solvent}}{\text{weight of seaweed}}$$

2.3 2, 2-diphenyl-1-picrylhydrazyl radical scavenging

The DPPH free-radical scavenging experiment was

https://doi.org/10.26656/fr.2017.7(S4).4

carried out by using the method described by Chew *et al*. (2008) in triplicates. The reaction mixture was made up of 1 mL extract and 2 mL of 0.15 mM DPPH (2,2-Diphenyl-1-picrylhydrazyl from Aldrich, Germany), and after 30 mins it was measured at 517 nm for absorbance. The concentration of both samples ranged from 4-20 mg/mL. The DPPH inhibition (IC50%) and ascorbic acid (R and M Chemicals, UK) equivalent were derived using the following equation follows:

$$\% \text{ Scavenging activity} = \left[\frac{Ac - As}{Ac}\right] x\ 100\%$$

Where Ac = absorbance of the control, As = absorbance of the sample solution.

AEAC (mg AA/100 g) = IC50 (ascorbate) / IC_{50} (sample) × 100,000

2.4 Ferric-reducing antioxidant power

Determination of the FRAP assay was following the method used by Hossain *et al*. (2021) and was conducted in triplicates. A mixture of 1 mL of diluted extracts and 1.25 mL 0.2 M potassium phosphate buffer (pH 6.6) were mixed thoroughly. 1.25 mL 1% potassium ferricyanide (Bendosen, Malaysia) was added then (w/v) and the mixture was vortexed and incubated (Memmert Incubator Oven, Germany) for 20 mins at 50°C. About 1.25 ml of 10% w/v trichloroacetic acid (Sigma Aldrich, USA) was added to the reaction mixture and centrifuged for 10 mins at 3,000 rpm. The subsequent step involves separating about 1.25 mL of the supernatant was separated and 1.25 mL of distilled water and 0.25 ml of 0.1% w/v $FeCl_3$ (Iron (III) Chloride Hexahydrate from R and M Chemicals, UK) were added to the reaction mixture. The solution was incubated at room temperature for 10 mins to allow for colour development. The absorbance then is calculated at 700 nm. The FRAP value was computed in the mg GAE/100 g sample and mg TE/100 g sample (Trolox 97% from Acros Organics, USA), by using the following equation:

$$\text{FRAP value} = \frac{\text{raw FRAP value x dilution factor x volume of the solvent}}{\text{weight of seaweed}}$$

The calibration equation of gallic acid was y = 0.0211x±0.0151 (R^2 = 0.9862) while Trolox gave calibration equation y = 0.0043x±0.0022 (R^2 = 0.9974). Both gallic acid and Trolox concentrations used were in the range of 0-20 mg/L.

2.5 Flavour profile analysis

SPME procedure was conducted by using Supelco, SPME Fibre Assembly 65 µm PDMS/DVB according to Norakma *et al*. (2022) and Madihah *et al*. (2012) with slight modification. About 0.5 g solid sample was placed into a 15 mL headspace vial. After conditioning the steel needle, the fibre was pushed through the vial septum. The extraction process was exposed to the headspace at 80°C for 15 mins (Hotplate Stirrer from Saintifik Maju, Malaysia) before injection at the GC port for the thermal desorption analysis. Before the change of different samples and after being analysed, the SPME should be conditioned at 250°C for 15 mins at the injection port.

The 7890A GC system from Agilent Technologies, which has a 5975C Triple-Axis Detector Mass Selective Detector was utilised to analyse the volatiles (Zaibunnisa *et al*., 2016). The column used was HP-5MS 5% Phenyl Methyl Silox (30 m × 250 µm × 0.25 µm). Flow rate, pressure, average velocity and hold time were 1 mL/min, 7 psi, 36.262 cm/sec and 1.3789 min at constant flow, respectively. Helium was used as the carrier gas in splitless mode. The oven temperature program was initialized at 40°C and held for 2 mins before increasing (with a rate of 5°C /min) to 150°C and held for 5 mins. Then increased at a rate of 10°C/min until reached 220°C and held for 10 mins. The compounds were recognised by comparing their mass spectral fragmentation patterns and relative retention indices to those of the NIST data stored in the instrument's internal library. The samples were analysed in triplicate.

2.6 Sensory and satiety analysis

Thirty untrained panelists employed the 9-Point Hedonic Scale to determine which formulation is the most accepted (1 = extremely dislike, 9 = extremely like). Table 1 shows the formulation used in the preparation of oat drinks. As mentioned in the packaging, 4.7 g of oats were put into a cup, followed by ±25 mL of boiling water. 30 panelists evaluated the oat drinks with a 5 mL sample prepared after 1 min of agitation. The attributes that have been evaluated are the textural characteristics including both physical (thickness) and oral (homogeneity, slipperiness and coarseness) characteristics (Lapveteläinen and Rannikkainen, 2000). The panelists for the satiety test were among the staff and students between the ages of 22 and 51 and comprises both males and females. The panelists were given a questionnaire related to hunger, fullness, and desire to eat. The scale ranges from 1 to 10 (for hunger; 1 is not at all and 10 is extremely hungry, for fullness; 1 is not at all and 10 is full, and for wants to eat; 1 is not at all and 10 is very eager to eat) were determined pre- and -post-meal of Formulation 4 (the highest amount of SSF) according to the method used by

Table 1. Formulation of instant oats drinks.

Ingredients (%)	Control	F1	F2	F3
Instant oatmeals	100	100	100	100
Sugar (ratio to oats)	2	2	2	2
Fermented *C. racemosa*	-	0.25	0.5	0.75

https://doi.org/10.26656/fr.2017.7(S4).4

Forde (2018) and the Visual Analog Scale is presented in Figure 1.

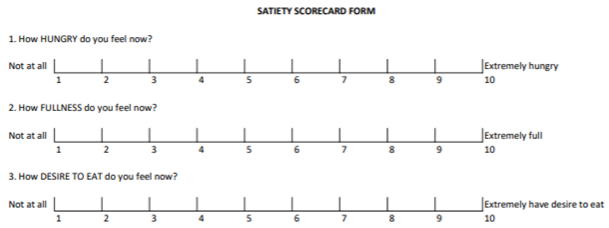

Figure 1. Visual analogue scale (VAS) for satiety assessment.

2.7 Statistical analysis

Data analysis was carried out using ANOVA Variance analysis (SPSS, ANOVA) version 28.0.0.0 (190).

3. Results and discussion

3.1 Effects of solid-state fermentation on antioxidant assay

Among the wide variety of food products that have been created, those made by solid-state fermentation (SSF) provide a quick and affordable way to enhance the nutritive content and sensory qualities (Suraiya *et al*., 2018; Ayyash *et al*., 2019). Besides plants are provide several free phenolic compounds or known as soluble, and they also present in the bound form (Gan *et al*., 2019). In plants, the intracellular endoplasmic reticulum produces and stores the majority of the soluble phenolic chemicals. However, soluble phenolic compounds are transported to the cell wall where they are converted into bound phenolic compounds via ester and glycosidic linkages with the help of cell wall macromolecules like cellulose and protein. The availability of the bound phenolics originally from the plant is limited due to the bond of sugar molecules and hydroxyl groups in glycosides will be broken by the solid-state fermentation (Abdel-Aty *et al.,* 2022) and at the same time increasing the phenolic compounds. The comparisons in polyphenols and any antioxidant activities between unfermented and SSF *C. racemosa* are shown in Table 2.

The TPC of unfermented *C. racemosa* was significantly ($P<0.05$) lower than that of fermented *C. racemosa*. The increase of TPC was about 113.66% from 146.17±7.61 (mg GAE/100 g samples) to 312.30±5.76 (mg GAE/100 g samples), respectively. These results correlate well with the previous study, the amount of unfermented *C. racemosa* TPC was 144±22 (Chew *et al*., 2008). Previous other researchers also obtained similar results, the TPC values of *C. racemosa* were 13.41±0.86 mg GAE/g (Yap *et al*., 2019), 17.88±0.78 mg GAE/g (Aroyehun *et al*., 2020), 19.711±0.2546 mgGAE/L (Sarini *et al*., 2014) and 27.43 mg GAE/100 g (Chia *et al*., 2015). However, some differences in values were observed which might be due to different extraction solvents, methods and origin of the samples used in their study. The unfermented result can be obtained by the measure of the free phenolic compounds present in the seaweed and the fermented result were carried out by the addition of phenolic compound from the breaking of the cell wall due to fermentation. Referring to Abdel-Aty *et al*. (2015), this result showed due to cellulose's Referring to decomposition since has been metabolized by microbes throughout the fermentation phase. These results correlate well with the finding, microbial metabolic activity during fermentation may significantly alter the profiles of beginning phenol molecules, because of several distinct hydrolysis processes, resulting in the release of less complex phenolics from the plant tissues.

The free radical DPPH has often been used to assess reducing agents. A free radical scavenger may easily eliminate DPPH since it contains a nitrogen-free radical. This experiment determined the antioxidative chemicals' capacity to scavenge proton radicals (Sarini *et al*., 2014). The DPPH radical scavenger for SSF *C. racemosa* (25.96±0.17 mg AA/100 g) was significantly higher than unfermented *C. racemosa* (4.87±0.37 mg AA/100 g) or as high as 433.06% increased. The results obtained for IC50 value complemented these findings, whereby IC50 value for unfermented *C. racemosa* was significantly ($p<0.05$) higher, 247.40±18.79 mg/mL while in SSF *C. racemosa* was 46.22±0.31 mg/mL. These findings can be explained that the SSF *C. racemosa* has greater radical scavenging activity than unfermented *C. racemosa*. Furthermore, the SSF *C. racemosa* only need a less amount sample concentration to inhibit 50% of radical (18.68% sample compared to unfermented seaweed). A previous study showed the same pattern, the fermented *Laminaria japonica* needs only 57.17% compared to unfermented to inhibit 50% of radicals (Yue *et al*., 2021). This finding supported that the more effective a substance is in scavenging DPPH, and hence, the lower the IC50 value, the greater the antioxidant activity of the material (Sujarwo and Keim, 2019).

Table 2. The comparisons between unfermented and SSF *C. racemosa* in polyphenols and antioxidant activities.

	Unfermented *C. racemosa*	SSF *C. racemosa*
TPC (mg GAE/100 g)	146.17±7.61^{b}	312.30±5.76^{a}
IC_{50} (mg /mL)	247.40±18.79^{a}	46.22±0.31^{b}
DPPH (% scavenging activity) (mg AAE/100 g)	4.87±0.37^{b}	25.96±0.17^{a}
FRAP value (mg TE/100 g)	272.21±3.60^{b}	1106.72±21.82^{a}
FRAP value (mg GAE/100 g)	36.36±1.80^{b}	206.80±4.76^{a}

https://doi.org/10.26656/fr.2017.7(S4).4

The FRAP assay (ferric reducing antioxidant power) was used to study the potential benefits of medicinal plants since it measures total antioxidant capacity. While IC50 DPPH works as an inhibitor scavenger (single electron transfer), the FRAP analysis was utilized to investigate how foods' antioxidants are absorbed (by hydrogen atom transfer). In Table 2, the FRAP values of the SSF *C. racemosa* was highly significant compared to the unfermented *C. racemosa* for about (306.57% increase using Trolox Equivalent (TE) standard curve and a 468.76% increase using Gallic Acid Equivalent (GAE) standard curve). The higher FRAP value indicates that high antioxidant potential in the SSF *C. racemosa* sample. Other than that, in the use of standard antioxidants, its antioxidative reaction is greater than that of Trolox. Gallic acid is widely employed as the standard antioxidant because it provides the greatest reaction among all evaluated standard chemicals in several applications (Olszowy-Tomczyk, 2021). Furthermore, GAE gave the highest values than TE, while the fermented *C. racemosa* remain the same result which was showing the highest FRAP value.

The presence of phenolic compounds in seaweed may be responsible for its capacity to convert the ferric cyanide complex into ferrous form in the FRAP value and the DPPH radical scavenging activity. In addition to polyphenols being secondary metabolites, (Makarewicz *et al*., 2021) that will be formed after the microbial growth stage of *A. oryzae*, cell wall destruction by fermentation during SSF was the cause of the augmentation of polyphenols and antioxidant activity. Different kinds of enzymes are created by bacteria during SSF. The release of conjugated phenolics because of cell wall degradation by microbial enzymes generated during SSF is primarily what allows SSF to increase the polyphenols and antioxidant activity of *K. alvarezii* (Norakma *et al*., 2019), releasing or producing several bioactive compounds of whole grain cereals (Adebo and Gabriela Medina-Meza, 2020).

3.2 Flavour profile analysis in the unfermented and solid -state fermentation Caulerpa racemosa

Taste release is the movement of flavour molecules from a specific molecular environment of a meal into the surrounding saliva or vapour phase (McClements, 2009) and depends on the mechanisms that govern the volatilization of aroma molecules from meals in a variety of settings, including formulation, processing, storage, and consumption (Voilley and Souchon, 2006). Since most flavour and aroma chemicals are volatile, GC is the preferred approach. Using 90% similarity, as seen in Table 3, the comparison analysis of volatile compounds contribution in unfermented and SSF *C. racemosa*. There was an increasing area of siloxane group which are hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, decamethylcyclopentasiloxane, dodecamethylcyclohexasiloxane, tetradecamethylcycloheptasiloxane, and butylated hydroxytoluene during fermentation. The primary phyco-constituents were discovered by the GC-MS to be hexamethylcyclotrisiloxane and octamethylcyclotetrasiloxane. It is conceivable to suggest that bioactive compounds largely composed of hexamethylcyclotrisiloxane may be engaged in biological activity as the GC-MS analysis can only be able to cover non-polar and volatile components from the analysed extracted samples (Bhuyar *et al*., 2020). It has been observed that the cyclic, unsaturated cyclotrisiloxane, hexamethyl (22.36%), octamethylcyclotetrasiloxane (26.84%), decamethylcyclopentasiloxane (18.60%), and dodecamethylcyclohexasiloxane (10.63%) exhibits antimicrobial action, antibacterial activity, an antioxidant activity which is essential in the removal of free radicals from the body (Ismail *et al*., 2020). This result was matched with the previous study of *S. tenerrimum* major compounds, which was cyclotrisiloxane hexamethyl (Sowmiya *et al*., 2016) and in GC-MS analysis of the acetone extract of *Turbinaria decurrens* (Ismail *et al*., 2020).

While ketone and the most alkane group disappear after fermentation. In instances, 2,2,6-Trimethylcyclohexane-1,4-dione, phytane, 3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, semicarbazone, 2,6,10-trimethyltridecane, pentadecane, hexadecane, 8-Heptadecene, phytone and nonadecane. The rest, heptadecane and 1-heptadecene were decreased after fermentation. And for octadecane which suddenly appears after fermentation. The result was correlated with the previous study n (Peppard and Halsey, 1981), while some saturated and non-conjugated unsaturated ketones were only partially reduced, aldehydes and vinyl ketone were chemically reduced during fermentation and could not be found in the finished beer. Octadecane appeared as a bacterial and plant metabolite and has antimicrobial activity (Rouis-Soussi *et al*., 2014; Alqahtani *et al*., 2022).

3.3 Sensory and satiety analysis in the incorporation of SSF Caulerpa racemosa in oat drinks

A sensorial analysis table is presented in Table 4. Generally, in terms of overall acceptability, panellists prefer formulation F3 (7.10). However, statistically, there were no significant differences ($P>0.05$) between formulations for all the attributes.

The satiety evaluation result can be seen in Table 5. The satiety test was used to compare the value of hungry,

https://doi.org/10.26656/fr.2017.7(S4).4

Table 3. The comparison analysis of volatile compounds contribution in unfermented and SSF *C. racemosa*

No.	RT in Chromatograph	Volatile compounds	Unfermented *C. racemosa*		SSF *C. racemosa*	
			Area	Area (%)	Area	Area (%)
1	5.745	Hexamethylcyclotrisiloxane ($C_6H_{18}O_3Si_3$)			18214834	22.36
2	5.785	Hexamethylcyclotrisiloxane ($C_6H_{18}O_3Si_3$)	21860549	7.95		
3	10.666	Octamethylcyclotetrasiloxane ($C_8H_{24}O_4Si_4$)	20189929	7.34		
4	10.674	Octamethylcyclotetrasiloxane ($C_8H_{24}O_4Si_4$)			21864526	26.84
5	15.311	Decamethylcyclopentasiloxane ($C_{10}H_{30}O_5Si_5$)	16372139	5.96		
6	15.319	Decamethylcyclopentasiloxane ($C_{10}H_{30}O_5Si_5$)			15151019	18.60
7	15.552	2,2,6-Trimethylcyclohexane-1,4-dione ($C_9H_{14}O_2$)	4564107	1.66		
8	20.105	Dodecamethylcyclohexasiloxane ($C_{12}H_{36}O_6Si_6$)	7471812	2.72		
9	20.112	Dodecamethylcyclohexasiloxane ($C_{12}H_{36}O_6Si_6$)			8661503	10.63
10	21.311	Phytane ($C_{20}H_{42}$)	7750643	2.82		
11	22.679	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, semicarbazone ($C_{14}H_{23}N_3O$)	2182717	0.79		
12	23.476	2,6,10-Trimethyltridecane ($C_{16}H_{34}$)	10970893	3.99		
13	24.427	Pentadecane ($C_{15}H_{32}$)	6595333	2.40		
14	24.474	Tetradecamethylcycloheptasiloxane ($C_{14}H_{42}O_7Si_7$)	10033235	3.65		
15	24.484	Tetradecamethylcycloheptasiloxane ($C_{14}H_{42}O_7Si_7$)			5749592	7.06
16	24.807	Butylated Hydroxytoluene ($C_{15}H_{24}O$ or $C_6H_2(OH)(CH_3)(C(CH_3)_3)_2$)	2752361	1.00		
17	24.813	Butylated Hydroxytoluene ($C_{15}H_{24}O$ or $C_6H_2(OH)(CH_3)(C(CH_3)_3)_2$)			7311240	8.97
18	27.505	Hexadecane ($C_{16}H_{34}$)	4255199	1.55		
19	30.412	8-Heptadecene ($C_{17}H_{34}$)	51424733	18.70		
20	31.033	Heptadecane ($C_{17}H_{36}$)	89362587	32.50		
21	31.053	Heptadecane ($C_{17}H_{36}$)			1633953	2.01
22	33.215	Octadecane ($C_{18}H_{38}$)			2199127	2.70
23	34.016	Phytone ($C_{18}H_{36}O$)	11789054	4.29		
24	34.04	1-Heptadecene ($C_{17}H_{34}$)			681713	0.84
25	34.447	1-Heptadecene ($C_{17}H_{34}$)	5774370	2.10		
26	34.815	Nonadecane ($C_{19}H_{40}$)	1580199	0.57		

Table 4. Sensory analysis of SSF *C. racemosa* in oat drinks formulation.

Attributes	Formulations			
	F1	F2	F3	F4
Viscosity	6.00±2.12[a]	5.80±2.07[a]	6.63±1.96[a]	6.17±2.05[a]
Adherence	6.67±1.86[a]	6.47±1.89[a]	6.93±1.70[a]	6.47±1.85[a]
Average Size of Swollen Flake Particles	6.40±1.63[a]	6.37±1.59[a]	6.47±1.85[a]	6.10±1.88[a]
Uniformity of Mass	6.07±1.68[a]	5.97±1.90[a]	6.4±1.83[a]	6.23±2.03[a]
Slipperiness	6.13±2.08[a]	6.27±1.55[a]	6.67±1.71[a]	6.27±2.00[a]
Coarseness	6.00±2.02[a]	6.03±1.90[a]	6.43±1.63[a]	5.80±2.14[a]
Odour	6.60±2.09[a]	6.67±2.17[a]	6.93±1.96[a]	6.90±1.86[a]
Flavour	6.03±2.21[a]	6.47±1.76[a]	6.67±2.17[a]	6.77±2.16[a]
Overall	6.53±1.87[a]	6.67±1.54[a]	7.10±1.69[a]	6.90±1.90[a]
Appetite Effect	6.13±2.05[a]	6.17±1.88[a]	6.53±2.00[a]	6.63±2.19[a]
Contribution of Fullness	6.50±1.90[a]	6.37±1.67[a]	6.70±1.77[a]	6.30±2.02[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

Table 5. Satiety analysis of SSF *C. racemosa* in oat drinks formulation F3.

Attributes	Before Treatment	After Treatment
Hungry	6.63±2.55[a]	5.80±1.90[a]
Fullness	4.50±2.49[b]	5.80±2.07[a]
Desire to Eat After	7.40±2.27[a]	6.80±1.75[a]

Values are presented as mean±SD. Values with different superscripts within the same row are statistically significantly different ($p<0.05$).

https://doi.org/10.26656/fr.2017.7(S4).4

fullness and desire to eat before and after consuming the formulation F3. Satiety score was used with a scale of 1 (indicates no at all) to 10 (very hungry/very full/very desired to eat). The results can be seen by comparing the mean of each pair. The mean of hungry value before the test was 6.63, then turned to 5.80, which indicated decreasing in the mean, which has the effect of reducing hunger after consuming the sample. At the satiety level, the initial mean was 4.50, and the final mean was 5.80, this shift indicates that the test administration gave the impression or feeling of fullness after consumption of the sample. The rate of desire to eat gives an initial mean of 7.40, which suggests the urge to eat decreases after consumption with a 6.80. So that the given sample gives the effect of reducing hunger, increasing satiety and reducing the desire to eat again. Thus, these results were compared using the two-way ANOVA p-value. Of the three pairs (hunger, fullness and desire to eat), which gives the level of correlation and significance is almost entirely, but the main one is from the level of fullness. This result is in line with the previous study that increasing dietary fibre consumption encourages fullness and reduces appetite. When combined with dietary fibre, oats lead to a longer-lasting sensation of fullness and a reduction in the consumption of meals heavy in fat or sugar. Moreover, oats also contain more protein per gramme than other cereals (Slavin and Green, 2007). In addition to being frequently indigestible, the seaweed carbohydrates (polysaccharides) that are isolated from specific seaweed species serve as soluble dietary fibres. Additionally, polyphenols lower blood sugar levels after meals heavy in carbs because they obstruct the action of the amylase and sucrase enzymes, which are necessary for the digestion and absorption of carbohydrates. Furthermore, it provides advantageous physiological benefits including enhanced satiety (Forster and Radulovich, 2015).

4. Conclusion

This study revealed that the SSF *C. racemosa* has higher TPC and antioxidant activity properties than the unfermented *C. racemosa*. The fermentation process breaks the cell wall and releases in-bound phenolics. Thus, as a consequence, it significantly enhanced the enormous amount of antioxidant properties of SSF *C. racemosa*. These findings were supported by the flavour profile analysis, with the increase of hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, decamethylcyclopentasiloxane, and dodecamethylcyclohexasiloxane after fermentation, which is role as antimicrobial, antibacterial and antioxidant. The incorporation of SSF *C. racemosa* in the oat drinks gave the fullness effect; no distinguish attributes can be detected compared to the control. This suggests that incorporating SSF *C. racemosa* in oat drinks may contribute functional value and satiety in terms of fullness without any effect on sensorial acceptance.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The research work was funded by UiTM internal grant (600-RMC/LESTARI SDG-T 5/3 (163/2019)) and also supported by the Institute of Educational Development-West Nusa Tenggara LPPNTB (184.LPPNTB.X.2022) under the NTB Province (Indonesia) Scholarship.

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