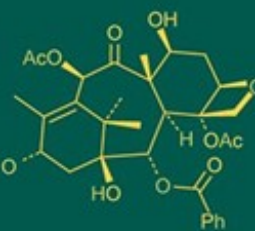
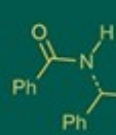


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Development and standardization of seaweed cookies incorporated with *Ulva fasciata* powder and alkali extract

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ABSTRACT

The present study was undertaken to develop and standardize cookies incorporated with *Ulva fasciata* powder and alkali extract as value-added bakery products. Fresh *U. fasciata* collected from the Ratnagiri coast was processed into dried powder and a freeze-dried alkali extract. The prepared seaweed ingredients were incorporated into cookie formulations at different levels and evaluated for sensory acceptability using a nine-point hedonic scale. A semi-trained panel of 30 members evaluated the developed cookies for appearance, colour, aroma, texture, taste, crispiness, mouthfeel, aftertaste and overall acceptability. In addition to seaweed ingredients, fat source and sugar level were standardized based on sensory evaluation. Among the *U. fasciata* powder treatments, 10% incorporation recorded the highest overall acceptability score of 8.60 ± 0.18 and was selected as the standardized level. For the alkali extract, 4.5% incorporation showed the highest overall acceptability of 8.50 ± 0.32 . Among the fat sources evaluated, ghee showed the highest sensory acceptability, with 4.5% incorporation recording an overall acceptability of 8.90 ± 0.32 , compared with butter and dalda. Sugar incorporation at the selected level also resulted in high sensory acceptance, with an overall acceptability score of 8.90 ± 0.22 . The observed differences among the standardization treatments were statistically significant ($p < 0.01$). The study demonstrates the feasibility of utilizing *U. fasciata* powder and alkali extract as functional marine ingredients in cookie development. The standardized formulations provide a basis for further evaluation of the physicochemical, nutritional, textural, colour and storage characteristics of seaweed-enriched cookies.

Keywords: *Ulva fasciata*, seaweed cookies, alkali extract, seaweed powder, bakery product, sensory evaluation, standardization, functional food

1. Introduction

Seaweeds are marine macroalgae that are increasingly recognized as valuable sources of nutrients, dietary fibre, minerals and biologically active compounds with potential applications in the food industry. They are broadly classified into green, red and brown algae, with the genus *Ulva* representing an important group of green seaweeds. Seaweeds are particularly attractive as functional food ingredients because their nutritional composition includes minerals, polysaccharides, proteins and various bioactive compounds, while their composition varies according to species, geographical location, season and environmental conditions. The nutritional and functional characteristics of seaweeds have stimulated increasing interest in their incorporation into conventional food products. Seaweed-derived polysaccharides and other bioactive constituents have been associated with dietary fibre and several biological activities, while seaweeds can also contribute minerals and other micronutrients to the diet. Consequently, their incorporation into commonly consumed foods represents a promising approach for developing value-added and functional food products. However, the utilization of seaweed in food formulations requires appropriate processing and optimization because excessive incorporation may adversely affect sensory properties such as colour, flavour, texture and overall acceptability.

Ulva fasciata, a green seaweed, has potential as a food ingredient because *Ulva* species contain nutritionally and functionally important components. The cell-wall matrix of seaweeds, however, can restrict the release and utilization of some constituent compounds. Therefore, processing techniques that improve the availability of valuable components are of considerable importance in developing seaweed-based food ingredients. Among the different extraction approaches, alkaline extraction has been reported as an effective method for improving protein recovery from seaweeds. Alkaline conditions can facilitate disruption of interactions be

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tween proteins and polysaccharide components of the algal cell wall, thereby enhancing the solubilization of proteins and other extractable constituents.

The form in which seaweed is incorporated into a food product may influence its nutritional, physicochemical and sensory characteristics. Direct incorporation of dried seaweed powder provides the advantage of utilizing the whole biomass, including its fibre and mineral fractions, whereas incorporation of an extract may provide a more processed ingredient containing soluble and extractable components. Thus, comparing seaweed powder with an alkali extract can provide useful information regarding their suitability as functional ingredients and their effects on the characteristics of the final food product.

Bakery products, particularly cookies, offer a suitable vehicle for the incorporation of functional ingredients because they are widely consumed and can accommodate different nutritional ingredients without requiring major changes in conventional processing practices. Previous studies have demonstrated that incorporation of seaweed into cookies can improve nutritional attributes, particularly protein, dietary fibre and mineral content, while maintaining acceptable sensory characteristics when the level of incorporation is appropriately optimized. However, increasing the proportion of seaweed beyond an optimum level may negatively influence sensory acceptability.

Although considerable attention has been given to the incorporation of seaweed biomass into food products, comparative information on the utilization of *Ulva fasciata* in powder and alkali-extracted forms for cookie development remains limited. The differences in the physical and functional characteristics of these two ingredient forms may influence dough properties, baking characteristics, nutritional composition and sensory quality of cookies. Therefore, systematic standardization is necessary to identify a suitable level and form of *U. fasciata* incorporation while maintaining desirable product quality.

Accordingly, the present study was undertaken to develop and standardize cookies incorporated with *Ulva fasciata* powder and alkali extract and to evaluate their quality characteristics. The study provides an approach for the value addition of an underutilized marine bioresource through its incorporation into a familiar bakery product and contributes to the development of seaweed-based functional food products.

2. Materials and Methods

2.1 Materials

Fresh *Ulva fasciata* was collected from the rocky intertidal region of the Ratnagiri coast, Maharashtra, India, during low tide. The collected seaweed was washed thoroughly with seawater to remove adhering sand, debris and epiphytic materials and was subsequently washed with distilled water in the laboratory. Whole wheat flour, powdered sugar, butter, skim milk, baking powder and egg yolk used for cookie preparation were of commercial food-grade quality and were procured from the local market.

2.2 Preparation of *Ulva fasciata* powder

The cleaned *U. fasciata* samples were dried at room temperature (22–30 °C) for three days to reduce moisture content. The dried seaweed was packed in zip-lock covers and stored under dark conditions. The dried biomass was subsequently ground using a mixer grinder to obtain powder, with the prepared material reported in the particle-size range of approximately 500–2000 µm. The powder was packed in zip-lock covers and stored at room temperature under dark conditions until further use.

2.3 Preparation of alkali extract of *Ulva fasciata*

The alkali extract of *U. fasciata* was prepared using a modified sodium hydroxide (NaOH)-based extraction procedure. A 7.5 M NaOH stock solution was prepared as Solution A. The working alkali solution was prepared by diluting 200 mL of Solution A with 800 mL distilled water to obtain a final volume of 1 L. The seaweed biomass was subjected to preliminary soaking for either 12 or 24 h, with or without supplementary ultrasonication, depending on the extraction

treatment. Subsequently, 10 g of the soaked seaweed sample was treated with the working alkali solution and incubated for 24 h at room temperature. The mixture was then autoclaved, cooled and centrifuged, and the resulting supernatant was neutralized to pH 7.0. The neutralized extract was subsequently freeze-dried and stored at 4 °C in amber-coloured vials until further use.

Important manuscript point: the synopsis describes four alkali extraction treatments, AIE-1 to AIE-4, but does not explicitly state in the visible methodology which one was finally selected for cookie formulation. The paper should therefore insert the actual selected extraction code rather than assuming one. The synopsis does establish that *U. fasciata* and particularly its alkali extraction were selected for product development based on the comparative evaluation.

2.4 Experimental design and standardization of cookies

Two forms of *U. fasciata*, namely whole seaweed powder and freeze-dried alkali extract, were used for cookie development. Each ingredient was incorporated at five levels: 4.5, 5.5, 7.5, 10.5 and 12.5% (w/w). A control formulation without any seaweed-derived ingredient was maintained for comparison. The incorporation level represented replacement of an equivalent proportion of whole wheat flour in the basic cookie formulation. The formulations were prepared in randomized order with three independent baking replicates for each treatment level.

For clarity in the manuscript, the treatments may be designated as:

Treatment	Ingredient	Incorporation level (%)
C	No seaweed ingredient	0
UP1–UP5	<i>U. fasciata</i> powder	4.5, 5.5, 7.5, 10.5, 12.5
AE1–AE5	<i>U. fasciata</i> alkali extract	4.5, 5.5, 7.5, 10.5, 12.5

These treatment levels are directly based on the experimental design described in the synopsis.

2.5 Preparation of *Ulva fasciata*-enriched cookies

The basic cookie dough was prepared using whole wheat flour (100 g), powdered sugar (50 g), butter (50 mL), skim milk (40 mL) and baking powder (0.5 g), with egg yolk used as the binding agent. Butter and sugar were creamed initially, followed by incorporation of the remaining ingredients. The required quantity of *U. fasciata* powder or alkali extract was incorporated at the respective treatment level by replacing an equivalent proportion of whole wheat flour.

All ingredients were mixed thoroughly to obtain a homogeneous, firm dough. The dough was portioned into uniform one-tablespoon portions and placed on oil-greased baking trays. The cookies were baked in an oven at 160 °C for 30 min. After baking, the samples were cooled to ambient temperature, chilled at 4 °C for 30 min and sealed in polyethylene packaging prior to quality evaluation.

2.6 Sensory evaluation

Sensory quality of the developed cookies was evaluated using the 9-point Hedonic Scale according to Peryam *et al.* (1957) [1]. A semi-trained sensory panel comprising 30 panelists consisting of postgraduate students, faculty members and research scholars was used for evaluation. Panelists were briefed on the sensory evaluation procedure before the test.

The cookie samples, including the control and seaweed-incorporated formulations, were prepared under identical processing conditions and coded using randomly generated three-digit numbers. Samples were served in randomized order at room temperature under uniform environmental conditions. Drinking water was provided for palate cleansing between successive samples.

The sensory attributes evaluated were appearance, colour, aroma, texture, taste, crispiness, mouthfeel, aftertaste and overall acceptability. Scores were assigned on a scale from 1 to 9, where 9 represented “like extremely” and 1 represented “dislike extremely.”

2.7 Instrumental texture analysis

The textural characteristics of the cookies were evaluated using a Food Texture Analyzer. Cookie texture was determined by a fracture test, in which the probe moved downward at a constant speed until structural failure or breakage occurred. The resulting force–distance data were recorded and processed using the texture-analyser software to obtain the characteristic textural parameters.

2.8 Colour analysis

The colour of the cookie samples was measured in triplicate using a colourimeter. The parameters L^* , a^* and b^* were recorded to represent lightness, redness/greenness and yellowness/blueness, respectively. The total colour difference (ΔE) relative to the control was calculated as:

$$\Delta E = \sqrt{(L^* - L_c)^2 + (a^* - a_c)^2 + (b^* - b_c)^2}$$

where L^* , a^* , b^* represent the colour values of the treatment sample and L_c , a_c , b_c represent the corresponding colour values of the control.

2.9 Selection of standardized formulation

The optimum level of *U. fasciata* powder and alkali extract was selected based on sensory acceptability, particularly overall acceptability, along with the observed quality characteristics of the cookies. The formulation showing the desired sensory response was considered the standardized formulation for subsequent evaluation. The broader study selected *U. fasciata* for product development after comparative assessment of five seaweed species, with *U. fasciata*

showing the highest protein content, particularly under alkali extraction, together with favourable preliminary sensory acceptance.

2.10 Statistical analysis

The experimental data were subjected to statistical analysis to determine significant differences among treatments and incorporation levels. The original study used XLSTAT (2024 version) and GraphPad Prism v.10.6.1 for statistical analysis. A two-way analysis of variance considering treatment group and incorporation level was used to evaluate treatment effects and their interaction, followed by appropriate post-hoc mean separation where significant differences were observed.

3. Results

3.1 Standardization of ingredients for cookie preparation

All ingredients used for the preparation of cookies were standardized individually on the basis of sensory acceptability using a 9-point hedonic scale. The ingredients standardized for the present study were *Ulva fasciata* powder, *U. fasciata* alkali extract, ghee, butter, dalda and sugar.

3.1.1 Standardization of Ulva fasciata powder

Cookies were prepared by incorporating *U. fasciata* powder at 0, 2.5, 3.5, 4.5, 5.5, 7.5, 10 and 12.5% levels. Among the formulations evaluated, 10% *U. fasciata* powder (UPC6) recorded the highest overall acceptability score of 8.60 ± 0.18 . The difference among the treatments was statistically significant ($p < 0.01$). Therefore, 10% *U. fasciata* powder was selected as the standardized level for further cookie preparation.

Table 1. Standardized Recipe of Preparation of cookies from Ulva Powder

Ingredients (%)	C	UALC 1	UALC 2	UALC 3	UALC 4	UALC 5	UALC 6	UALC 7
		2.5	3.5	4.5	5.5	7.5	10	12.5
Maida	45.50	43.00	42.00	41.00	40.00	38.00	35.50	33.00
Seaweed Content	0.00	2.50	3.50	4.50	5.50	7.50	10.00	12.50
Ghee	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Powdered sugar	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Milk powder	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Baking powder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vanilla essence	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Milk	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Total	100	100	100	100	100	100	100	100

UALC = Ulva Algae Content

3.1.2 Standardization of Ulva fasciata alkali extract

Cookies were prepared with *U. fasciata* alkali extract at 0, 2.5, 3.5, 4.5 and 5.5% levels. The formulation containing 4.5% alkali extract (UEC3) exhibited the highest overall acceptability of 8.50 ± 0.32 and was selected for further formulation studies. The observed variation among treatments was statistically significant ($p < 0.01$).

3.1.3 Standardization of fat source

Three fat sources, namely ghee, butter and dalda, were evaluated at different incorporation levels. For ghee, cookies were prepared with 0, 2.5, 3.5, 4.5 and 5.5% incorporation. The 4.5% ghee formulation (FG3) recorded the highest overall acceptability of 8.90 ± 0.32 ($p < 0.01$).

For butter, incorporation levels of 0, 2.5, 3.5, 4.5 and 5.5% were evaluated. The 4.5% butter formulation (FB3) showed the highest overall acceptability of 8.50 ± 0.27 ($p < 0.01$).

Dalda was evaluated at 0, 2.5, 3.5, 4.5 and 5.5% levels. The 2.5% dalda formulation (FD1) recorded an overall acceptability of 7.80 ± 0.31 ($p < 0.01$). Among the three fat sources evaluated, ghee was selected for the final cookie formulation based on sensory acceptability.

3.1.4 Standardization of sugar

Sugar was standardized at 0, 2.5, 3.5, 4.5 and 5.5% incorporation levels. The formulation containing 4.5% sugar (SR3) recorded the highest overall acceptability of 8.90 ± 0.22 and was selected for further cookie preparation ($p < 0.01$).

Table 2. Standardized Recipe of Preparation of cookies from Ulva Extract, Agar, Alginate, Carrageenin

Ingredients (%)	C	SWC 1	SWC 2	SWC 3	SWC 4	SWC 5	SWC 6	SWC 7
		2.5	3.5	4.5	5.5	7.5	10	12.5
Maida	45.50	43.00	42.00	41.00	40.00	38.00	35.50	33.00
Ulva powder	0.00	2.50	3.50	4.50	5.50	7.50	10.00	12.50
Ghee	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Powdered sugar	22.00	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Milk powder	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Baking powder	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vanilla essence	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Milk	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Total	100	100	100	100	100	100	100	100

SWC = Seaweed cookies

Table 3. Standardization of Ghee Content in the Cookies

Ingredients (%)	C	FG1 (20%)	FG2 (22%)	FG3 (24%) ★ Best	FG4 (26%)
Maida	48.00	53.31	51.31	49.31	47.31
Ghee	25.32	20.0	22.0	24.0	26.0
Powdered sugar	23.21	23.21	23.21	23.21	23.21
Milk powder	2.11	2.11	2.11	2.11	2.11
Baking powder	0.84	0.84	0.84	0.84	0.84
Salt	0.53	0.53	0.53	0.53	0.53
Total	100	100	100	100	100

FG : Fat Ghee treatment: FG3 (24%)

Table 4. Standardization of Dalda Content in the Cookies

Ingredients (%)	C	FD1 (20%) ★ Best	FD2 (22%)	FD3 (24%)	FD4 (26%)
Maida	53.31	51.31	49.31	47.31	45.31
Ghee	25.32	0.0	0.0	0.0	0.0
Dalda	0.0	20.0	22.0	24.0	26.0
Powdered sugar	23.21	23.21	23.21	23.21	23.21
Milk powder	2.11	2.11	2.11	2.11	2.11
Baking powder	0.84	0.84	0.84	0.84	0.84
Salt	0.53	0.53	0.53	0.53	0.53
Total	100	100	100	100	100

FD : Fat Dalda treatment: FD1 (20%) |

Table 5. Standardization of Sugar Content in the Cookies

Ingredients (%)	C	SR1 (18%)	SR2 (20%)	SR3 (22%) ★ Best	SR4 (24%)
Maida	48.00	53.20	51.20	49.20	47.20
Ghee	25.32	25.32	25.32	25.32	25.32
Powdered sugar	23.21	18.0	20.0	22.0	24.0
Milk powder	2.11	2.11	2.11	2.11	2.11
Baking powder	0.84	0.84	0.84	0.84	0.84
Salt	0.53	0.53	0.53	0.53	0.53
Total	100	100	100	100	100

SR: Sugar treatment: SR3 (22%)

Table 6. Standardization of Maida Content in the Cookies

Ingredients (%)	C	M1 (45.5%)	M2 (43.5%)	M3 (41.5%) ★ Best	M4 (39.5%)
Maida	48.00	45.5	43.5	41.5	39.5
Ghee	25.32	27.81	29.81	31.81	33.81
Powdered sugar	23.21	23.21	23.21	23.21	23.21
Milk powder	2.11	2.11	2.11	2.11	2.11
Baking powder	0.84	0.84	0.84	0.84	0.84
Salt	0.53	0.53	0.53	0.53	0.53
Total	100	100	100	100	100

M : Maida treatment: M3 (41.5%) |

Table 7. Standardized levels of ingredients used for cookie preparation

Ingredient	Levels evaluated (%)	Standardized level (%)	Overall acceptability
<i>U. fasciata</i> powder	0, 2.5, 3.5, 4.5, 5.5, 7.5, 10, 12.5	10.0	8.60 ± 0.18
<i>U. fasciata</i> alkali extract	0, 2.5, 3.5, 4.5, 5.5	4.5	8.50 ± 0.32
Ghee	0, 2.5, 3.5, 4.5, 5.5	4.5	8.90 ± 0.32
Butter	0, 2.5, 3.5, 4.5, 5.5	4.5	8.50 ± 0.27
Dalda	0, 2.5, 3.5, 4.5, 5.5	2.5	7.80 ± 0.31
Sugar	0, 2.5, 3.5, 4.5, 5.5	4.5	8.90 ± 0.22

All reported standardization trials were statistically significant at $p < 0.01$.

Final standardized ingredients

Based on the sensory standardization, 10% *U. fasciata* powder, 4.5% *U. fasciata* alkali extract, 4.5% ghee and 4.5% sugar were selected for the final cookie formulations. Ghee was selected as the fat source in-

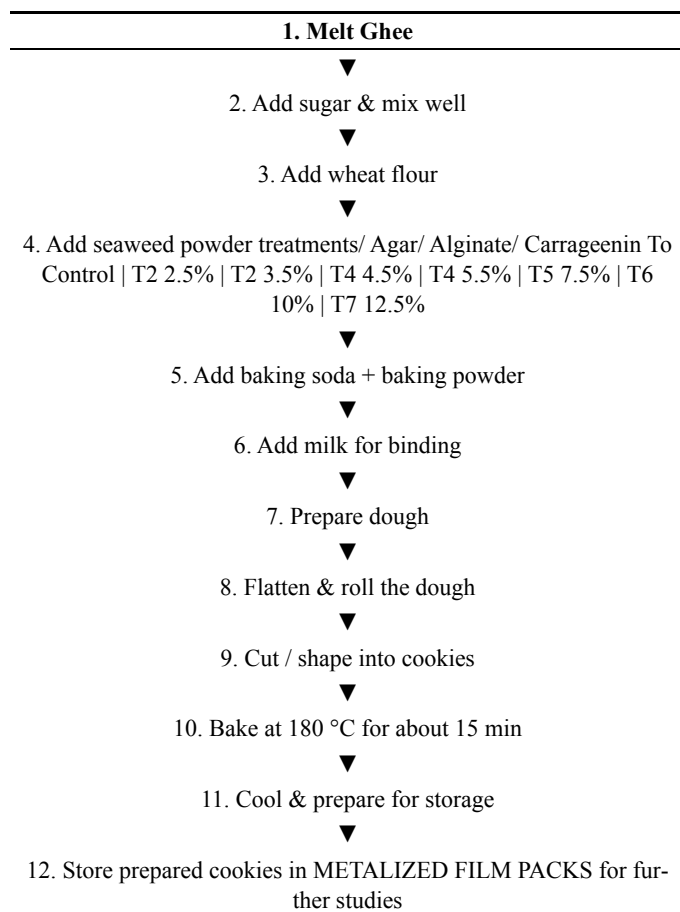
stead of butter or dalda because it recorded the highest sensory acceptability among the fats evaluated.

Table 8. Cookies Prepared by incorporating finger millet (Sinha, R., & Sharma, B. (2017))

Ingredient	Quantity
Refined wheat flour (maida)	50 g
Finger millet (ragi) flour	50 g
Butter	45 g
Powdered sugar	40 g
Milk	14–16 mL
Baking powder	1.0 g
Sodium bicarbonate	0.5 g
Salt / Vanilla	0.8 g / 0.5 mL

Table 9. Cookies Prepared by Local Recipe

Ingredient	Quantity
Refined wheat flour (maida)	40 g
Butter	24 g
Powdered sugar	22 g
Milk	20 – 22 mL
Baking powder	0.80 gm
Salt	0.5 g



Flowchart 1. Flow chart for preparation of seaweed-enriched cookies

4. Conclusion

The present study successfully demonstrated the potential of *Ulva fasciata* powder and alkali extract for incorporation into cookies as value-added marine food ingredients. Sensory standardization showed that the incorporation level had an important influence on the acceptability of the developed cookies. Among the powder treatments, 10% *U. fasciata* powder recorded the highest overall acceptability (8.60 ± 0.18), whereas 4.5% *U. fasciata* alkali extract recorded the highest overall acceptability (8.50 ± 0.32). The study further established suitable levels of the major formulation ingredients through sensory evaluation. Ghee was selected as the fat source over butter and dalda, while the standardized sugar treatment also exhibited high sensory acceptability. The highest overall acceptability scores recorded for ghee and sugar were 8.90 ± 0.32 and 8.90 ± 0.22 , respectively, and the differences among the evaluated treatments were statistically significant ($p < 0.01$). Overall, the findings indicate that *U. fasciata* can be incorporated into a familiar bakery product while maintaining desirable sensory characteristics when the incorporation level is appropriately optimized. The use of both whole seaweed powder and an alkali-derived ingredient also provides two different approaches for utilizing *Ulva* biomass in food product development. This is consistent with published research showing that *Ulva* species contain nutritionally relevant protein, dietary fibre, minerals and other bioactive constituents, while recent cookie studies have demonstrated the feasibility of incorporating seaweed into bakery products.

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