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Anethum Graveolens L. Microgreen Incorporated Pulse-Based Gluten-Free Crackers: A Potential Functional Snack

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Crackers were formulated with pulse flours – Chickpea and Pea along with the incorporation of Anethum Graveolens L. microgreens to develop an end product that is rich in protein, fibre, and micronutrients, gluten-free and low in glycemic index. Microgreens are tiny greens that are often harvested in an immature state that are loaded with innumerable benefits thus, Anethum Graveolens L. was utilized as a part of the study. The main goal was to develop a gluten-free snack that was rich in micronutrients due to the lack of availability of such products in the market. The crackers were inflicted to different analyses that included – sensory analysis, physicochemical analysis, proximate analysis, antioxidant analysis (DPPH Radical Scavenging Activity-DRSA and Ferric Reducing Antioxidant Power- FRAP) , texture analysis, microbial analysis, and shelf-life analysis. Results exhibited that the crackers had a remarkable consumer acceptance, were nutrient-dense, had potential antioxidant activity, minimal microbial activity, and had an extended shelf-life. The crackers weighed 3.0±1.0g per unit and the average pH of the crackers was 6.09±0.34. They were rich in protein (28.4g), dietary fibre (3.99g) micronutrients, high in antioxidants (DRSA-21.4% and FRAP-575.71 μM Fe(II)/g), and had low fat (9.19g) content per 100g of the crackers. Thus, the Anethum graveolens L. microgreen incorporated pulse-based gluten-free crackers are an ideal snack with phenomenal potential.

Keywords: Antioxidant; Cracker; Functional snack; Gluten-Free; Microgreen; Pulse-Based.

Microgreens are vegetable greens and herbs harvested before the plant's primary leaves appear. Microgreen consumption has soared in recent years as a consequence of higher concentrations of bioactive components in them than in mature greens, all of which are favourable to human health.[1–3] Several studies have been carried out reviewing the functional potential of the microgreens, where they are known to possess distinguished antioxidant properties, phenolic composition, and phytochemica

l composition, including the presence of chlorophyll, ascorbic acid, flavonoids, phenols, and lutein. [4–6] They are also acknowledged for their uses and benefits such as their ability to modulate weight gain and control, their protective

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mechanism against tumour cell proliferation, and other functions like anti-carcinogenic activity, anti-inflammatory effect, anti-diabetic effect, antimicrobial activity, anti-obesity effect, and capability to prevent malnutrition and chronic diseases. [7–10]

Gluten is a protein constituent that is fundamentally present in wheat and also in barley, rye, and oats in similar forms. [11,12] Gluten is not tolerated in patients having auto-immune disorders like celiac disease and non-coeliac gluten sensitivity or gluten intolerance, which when consumed results in gastric discomfort and other allergic reactions.

The gluten-free diet is by far the most effective therapy for celiac disease and gluten-related disorders. [13] Gluten-Free diets may lead to a possible nutrient imbalance resulting in the improper nutritional quality of diet, as there is only minimal presence of protein, dietary fibre, and micronutrients that lead to nutritional deficiencies and higher amount of saturated and hydrogenated fatty acids, also high-glycemic index is observed. [14] Patients with Celiac disease, gluten sensitivity, and/or health-conscious consumers are driving the economy for gluten-free bakery goods. Different gluten-free components are being used to produce similar bakery goods, although there have only been limited studies based on gluten-free biscuits, muffins, and crackers. [15]

Anethum graveolens L. (Dill) is a resilient parsley crop that grows every year. The leaves resemble ferns and are tiny and wispy. [16] The microgreens of *Anethum graveolens L.* were utilized as a part of the crackers due to their plausible benefits such as their micronutrient composition and their other acclaimed properties such as their anti-microbial, anti-diarrheal, anti-spasmodic properties, etc. [17]

The central aim of the study was to develop a gluten-free product, rich in dietary fibre, micronutrients, and protein as there is a necessity to develop a bakery product that is gluten-free and nutrient-dense. Two prominent pulses that were used as a part of the study are Chickpeas *(Cicer arietinum)* and Peas *(Pisum sativum L)* which have extensive nutrition profiles and a considerable amount of protein, antioxidant composition, and functional properties.[18,19]

The objectives of the study were to (a) to formulate a pulse-based gluten-free functional snack with the incorporation of *Anethum graveolens L.*microgreen (b) to evaluate the sensory acceptability and organoleptic characteristics of the crackers (c) to determine the physicochemical properties, microbial composition and shelf-life of the product.

MATERIALS AND METHODS

Materials

The raw materials required for the formulation of the crackers including – Chickpea, Peas, Dill seeds, Butter, and Salt were procured from the local supermarket. Equipment required for the study included an electronic weighing scale, measuring cups and spoons, baking equipment, a cooling tray, a sieve, mortar, an electronic vernier calliper, and other materials for the determination of the characteristics of the crackers obtained.

Pre-preparation of the Crackers

The process involved in the pre-preparation of the crackers are defined below for the microgreen and the pulses respectively.

Anethum Graveolens L. Microgreen

Cultivation

The procured *Anethum graveolens L.* seeds were utilized for cultivation. With minor adjustments, the seeds were sown and nurtured for 14 days as given by Weber, 2017 [20]for the soil/compost approach which attained full growth in a matter of 14 days that was used for further processing.

Drying

Microgreens were dried using an elemental methodology adopted from Naikwade, 2015 [21] and then processed with sun-drying at 90! for 2 days and microwaving for three minutes at 60°Clater they were coarsely ground in a mortar.

Pulses

Dry Roasting

Chickpeas and Peas are dry roasted for 15 minutes at 100°C, then cooled.

Milling

The dry roasted and cooled pulses are milled and processed using an electric grinder with a mesh of 0.42mm and grounded to a fine powder that was utilized for the formulation of the crackers.

Formulation of Crackers

The crackers were formulated with a basic

methodology derived from Han et al., 2010 [22]with modifications based on the current study.

All of the components were weighed using an electronic weighing scale before being combined into a dough. Kneading the flour mix with the 15g of butter, 2g of salt, and 50 -80ml water yielded the dough. In the case of the Control treatment of the crackers, 100% wheat flour was used as the flour mix. The crackers were formulated in six different variations as per the ratios given in Figure 1.

With the use of a round cookie cutter of 5 cm diameter, the dough was sheeted until the thickness was 1.50mm and cut into shapes. Before baking, the baking pan was smeared with butter and baked at 175°C for 15 minutes. The baked crackers are cooled and stored in an airtight container for further evaluation.

Sensory Evaluation

The Nine-Point Hedonic Scale was used to conduct the study's sensory analysis. [23] The experiment included 25 untrained panellists (17 women and 8 men) who belonged to the age group of 18-47 and tasted the various cracker varieties. The crackers were graded based on their appearance, texture, taste, flavour, mouthfeel, odour, and overall acceptability. The highly acceptable cracker was subjected to further evaluation.

Determination of Physicochemical Properties

Weight

The weight of the different variations of the crackers was recorded in triplicates with the help of an electronic weighing scale.

pH

The pH of the cracker was analyzed with the help of a pH meter.

Colour

Colour aspects of the cracker were investigated with the help of a Hunter Colorimeter that indicated the colour of the sample.

Water Activity

Water activity(a_w) aids in knowing how long the food can be preserved and consumed. a_w is measured with the help of a Water Activity Meter.

Determination of Proximate Composition

The composition of the following nutrients was analyzed – Energy, Protein, Carbohydrate, Fat, Ash, Moisture, Dietary Fibre, Iron, Potassium, Vitamin A, and Vitamin C. Methodology derived from AOAC was applied to analyze the composition of the following nutrients.

Determination of textural properties

Texture Analyzer was utilized that helps in determining the definitive texture of the particular food product which also aids in acknowledging its quality. [24] Texture Analysis was executed with the Texture Analyzer TA.XT Plus software and the probe used was a 75mm Compression platen. A P/75 Probe TPA test was conducted to analyze the textural properties of the crackers.

Determination of Antioxidant Properties

DPPH radical scavenging activity (DRSA) and Ferric Reducing Antioxidant Power (FRAP) were analyzed with the help of a UV Spectrophotometer.

DPPH radical scavenging activity (DRSA)

The DPPH radical scavenging activity (DRSA) was carried out by the following process as given by ***Riaz et al., 2012***[25]**.** The DPPH Radical Scavenging Activity was calculated by the formula:

DRSA (%) = [Absorbance of control – Absorbance of sample / Absorbance of control × 100

Ferric Reducing Antioxidant Power

FRAP assay was carried out by the method given by *Benzie & Strain,* 1999[26] and Rahim et al., 2017[27]. The FRAP value of the crackers was calculated with the help of the graph obtained. The FRAP values were expressed in µM Fe(II)/g

Determination of Microbial Composition And Shelf-life

The Total Plate Count (TPC) and the presence of Salmonella, Fungi, and Mould were performed on the sample in different packaging at room temperature at about 35°C to 37°C. The analysis for microbial composition was executed for a period of 45 days.

LDPE zip lock bags, vacuum containers, and aluminium foil boxes were used as packaging materials to determine the shelf-life at room temperature at about 35°C to 37°C. The crackers' quality was checked every three days for visible microbial growth, and changes in texture, flavour, and appearance were assessed using the Nine-Point Hedonic Scale.

Statistical Analysis

To interpret the data, statistical analyses

such as mean, median, standard deviation, and graphical representations were used as the study included minimal statistical analysis.

RESULTS AND DISCUSSION

The results of the various properties of the crackers are discussed below.

Sensory evaluation

The sensory characteristics of the crackers prepared in various variations, as well as the control group, were evaluated. The mean scores of the different cracker parameters were calculated and tabulated using the Nine-Point Hedonic Scale. The highest score was obtained by the variation A2 (90:10) which had overall acceptability of 8.79±0.45 which was higher than the overall acceptability of the control crackers which was 7.97±0.71. The A2 variation had the best acceptability in all the attributes and was thus proceeded for further evaluation.

Associating the sensory acceptability of the microgreen crackers to the ordinary pulse-based crackers that were studied by ***Han et al., 2010***[22], it was obvious that the sensory acceptability of microgreen crackers was higher than that of the control pulse-based crackers. The overall acceptability of chickpea crackers and pea protein isolate was 4.8 and 5.9 respectively as studied and presented by ***Han et al., 2010***[22], whereas the incorporation of the *Anethum graveolens L.* microgreen proved to elevate the consumer acceptability in great numbers.

Physicochemical Properties

Weight

The weight of the crackers was documented in triplicates and their mean weight was 3.0±1.0g.

pH

The average pH of the crackers was recorded to be 6.09±0.34 which thus denoted their slightly acidic and neutral nature.

Colour

The data was recorded in triplicates, for which the mean and standard deviation were obtained.

The acquired results indicate that the L* represents lightness in colour and the b* represents yellowness in colour, both of which were recorded at a higher level than the*, which represents redness in the sample. As a result, it's clearer to see that the crackers had a faint undertone even after adding microgreens.

Han., 2010[22] investigated the colour of crackers made from pulse flours and their fractions, focusing on crackers made from chickpea flour and pea protein isolate. The results of the investigation differed slightly from the results achieved with the microgreen crackers. The colour discrepancy can be attributed to differences in the cracker's processing time and baking temperature, as well as the addition of microgreens, which alter the cracker's colour.

Water Activity

The water activity (a_w) of the crackers was 0.247 at an ambient temperature of 32.5°C. The typical water activity of crackers varies between 0.1 and 0.3, depending on the cracker's components and contents. It is also clear that the microbial growth of many bacteria such as E.Coli, Salmonella species, Pseudomonas, and others is severely constrained since low water activity results in very little to no microbial activity. This also helps in enhancing the shelf life and the stability of the product. [28,29]

Proximal composition

The proximal compositions of the crackers are elaborated in Table 2.

AOAC Methods were utilized to obtain the nutritive composition of the crackers. The retrieved results indicated that:

Energy

The energy of the *Anethum graveolens L.* microgreen crackers was comparatively low than the control crackers as comprehended from the table.

Carbohydrate

Carbohydrate composition of the cracker was less than the control crackers as the *Anethum graveolens L.* microgreen crackers were exclusively pulse-based. The decline in carbohydrate content can be attributed to the usage of chickpea flour as well as losses incurred during processing. [32]

Protein

Remarkable results were observed in the case of the protein composition of the crackers as an approximate value of 28g of protein was found, which was almost two times higher than the control crackers. Chickpeas include a wide range of essential and non-essential amino acids

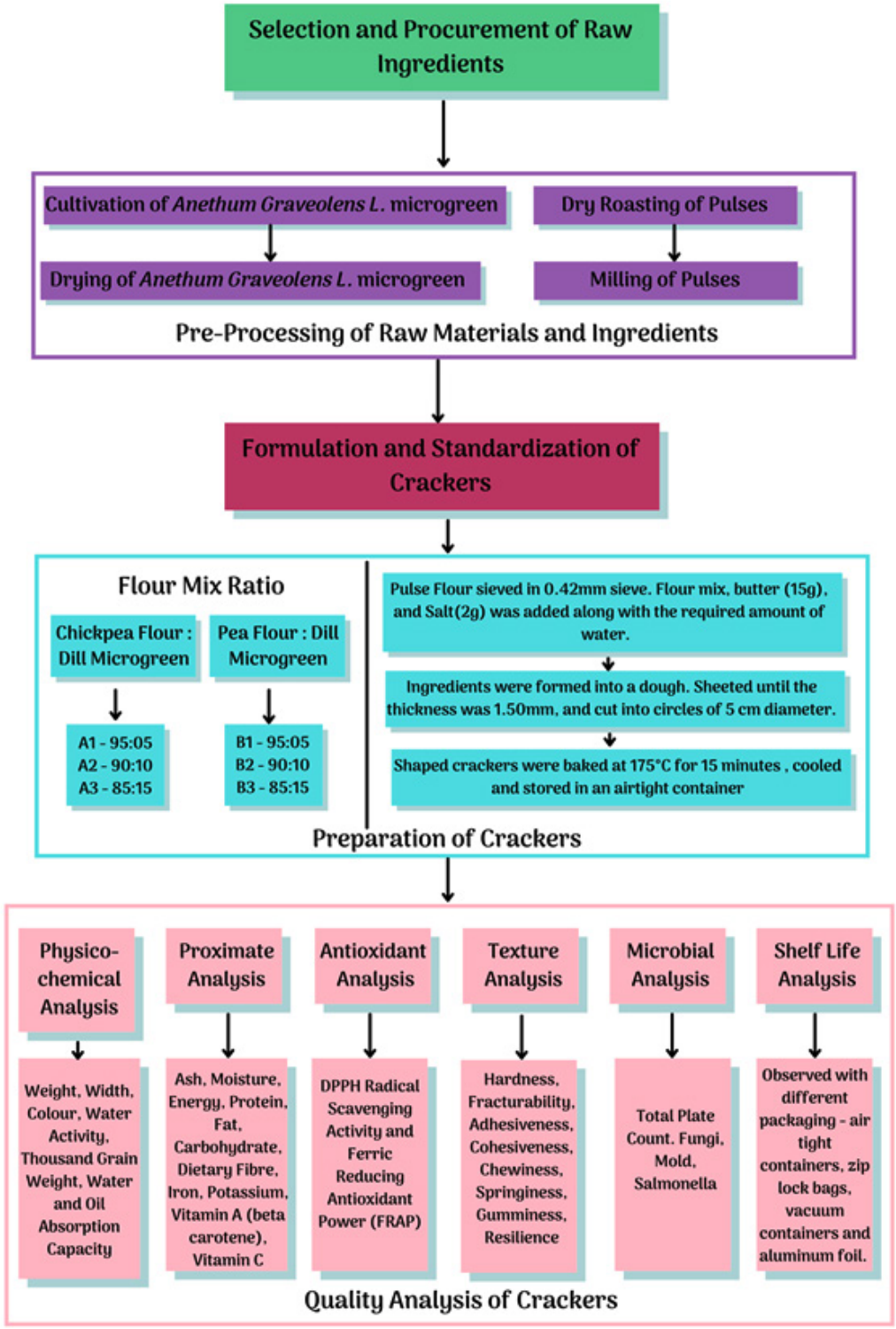

Fig. 1. The methodology followed for the study

that contribute to the protein content of crackers. [33,34]

Fat

Associating with whole wheat crackers or commercially available crackers, the fat content of the microgreen crackers was minimal. [30]

Dietary Fibre

The dietary fibre composition of the crackers was also phenomenal and was nearly ten times higher than the control crackers, thus providing 3.99g of dietary fibre. The inclusion of crude fibre in chickpea flour may also contribute to the enhanced dietary fibre in pulse-based crackers. [32]

Table 1. Amount of ingredients used in the crackers

Ingredients	Amount (g/ml)
Flour Mix (Pulse Flour and DriedMicrogreen)	100g
Butter	15g
Salt	2g
Water	50-80ml

Ash and Moisture

There was only minimal difference noticed in the case of the ash and moisture composition of the control crackers and the microgreen crackers. The discrepancy can be explained by differences in the cracker's components as well as the addition of microgreens in the crackers.

Iron

When compared to the control wheat crackers, the microgreen crackers prepared with chickpea flour had a much higher iron content as given by *FoodData Central, USDA*[31]. *Han et al., 2010*[22] found similar results in their study of the iron content in chickpea crackers, finding that the iron in chickpea crackers was 3 to 6 times greater than the various crackers that had been manufactured.

Potassium

In the case of potassium, the potassium levels of whole wheat and commercial crackers were significantly higher than those of microgreen crackers, yielding contradictory results. This massive disparity might be attributable to potassium loss during cracker manufacture, even though chickpeas are a fairly good source of potassium, according to the USDA. [31]

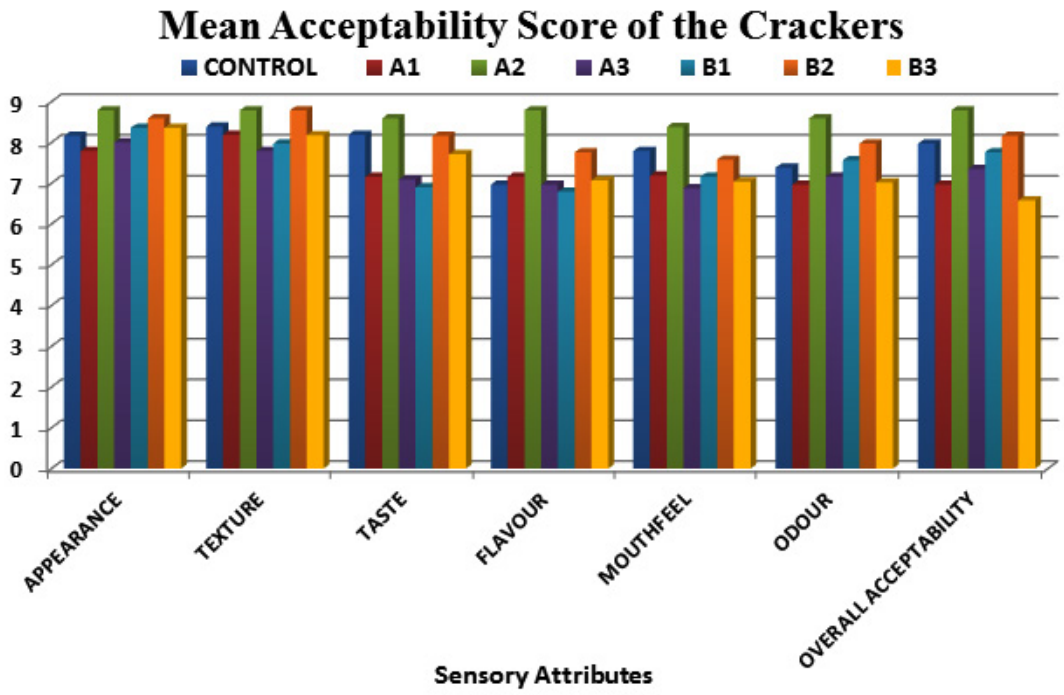

Fig. 2. Mean Scores of the Nine-Point Hedonic Scale explaining the consumer acceptability of the crackers

Vitamin A

The Vitamin A (â-carotene) levels of the crackers were found to be adequate. Whole wheat crackers and crackers made from pulses had no potential for delivering Vitamin A. [22,31]The presence of Vitamin A in the crackers can be justified due to the incorporation of microgreens in them although no specific data is prevalent for the Vitamin A composition in *Anethum graveolens L.* microgreen.

Vitamin C

Vitamin C was absent in whole wheat crackers or pulse-based crackers, just as it was in the case of Vitamin A. The presence of Vitamin C in the crackers has been enhanced by the incorporation of microgreens. [22,31] Hence, it can be understood that the microgreen incorporated pulse-based crackers have a better nutritional profile when compared to commercially available crackers, whole wheat crackers, or ordinary pulse-based crackers.

Table 2. Colour attributes of the cracker

Cracker Treatment	Colour Attribute L*	a*	b*	dE*
A2	59.18	8.37	38.46	50.56
	59.16	8.52	38.09	50.35
	57.79	6.77	34.12	48.40
MEAN±SD T1A2	58.71±0.65	7.89±0.79	36.98±1.96	49.77±0.97

Table 3. Proximate Composition of the crackers

Treatment Nutrient	Control (*-Benjakul & Karnjanapratum, 2018; #-FoodData Central, USDA)	A2 (*Anethum graveolens* L. microgreen cracker)
Energy (kcal)	471.38*	320.3
Carbohydrate (g)	67.03*	21.97
Protein (g)	11.53*	28.4
Fat (g)	17.64*	9.19
Dietary Fibre(g)	0.44*	3.99
Ash (%) w/w	3.97*	4.23
Moisture (%) w/w	2.94*	4.49
Iron (mg)	3.56#	24.04
Potassium (mg)	373#	32.45
Vitamin A (mcg)	0#	19.45
Vitamin C (mg)	0#	18.55

Table 4. Textural Properties of the crackers

Treatmenttexture Attributes	A2
Hardness (g f)	16718.64±8959.76
Fracturability (g f)	4456.16±2428.85
Adhesiveness (g sec)	-0.587±0.243
Springiness	0.36433±0.0611
Cohesiveness	0.51367±0.087
Gumminess	9365.41±6419.09
Chewiness	3803.02±3105.4
Resilience	0.42533±0.0887

Textural properties

A Texture Analyzer was used to examine the texture of the crackers, yielding the following results. The mean±SD value obtained for each texture attribute is discussed in the following section.

The crackers were subjected to texture analysis, which revealed that the crackers had favourable results in terms of hardness, fracturability, gumminess, and chewiness, but poor results in terms of adhesiveness. The samples

Table 5. Microbial Composition of the crackers

Microbial Test	A2 Day 0	A2 Day 3
Escherichia Coli	absent	absent
Salmonella species	absent	absent
Total Plate Count - Bacteria	$0.75x10^2$cfu/g	$1.25x10^2$cfu/g
Total Plate Count –Yeast, Fungi Mould	$0.35x10^2$cfu/g	$0.80x10^2$cfu/g

showed a steady level of springiness, cohesiveness, and resilience. As a result, the hardness of the crackers was shown to be far more ubiquitous than any other attribute.

ilmaz & Karaman, 2017[35] studied the textural attributes of functional crackers by incorporating the dietary fibre extracted from citrus seeds where the hardness and the fracturability of the crackers are studied. A remarkable difference was observed between the results obtained and the derived results from the study, but this helped in understanding the structural integrity of the crackers. Fracturability helps in comprehending that the crackers can crack and break easily whereas hardness helps in understanding the structural and textural nature of the cracker.

Correspondingly, another study by *Qadri et al., 2018*[36] studied the adhesiveness, springiness, and cohesiveness of crackers incorporated with brown rice flour and carboxymethyl cellulose. This study presented similar results as the textural attributes of the microgreen incorporated crackers and a negligible amount of difference was noted from the values obtained regarding the adhesiveness, springiness, and cohesiveness, where the crackers lacked in adhesivity and the minimal amount of its ability to retain a springy and cohesive nature. This aided in comprehending the textural attributes of the crackers.

Antioxidant properties

Antioxidants absorb free radicals and molecular oxygen, allowing them to halt or stop oxidation processes caused by molecular oxygen or reactive oxygen species. [37] To investigate the crackers' antioxidant capacity, two primary antioxidant assays were utilized, each with its mechanism. The DPPH Radical Scavenging Activity assay measures reactive oxygen species (ROS), whereas the Ferric Reducing Antioxidant Power (FRAP) assay measures redox potential. [38]

The results obtained are discussed based on the two different assessments:

DPPH Radical Scavenging Activity (DRSA)

The *Anethum graveolens L.* microgreen incorporated crackers portrayed a Radical Scavenging % of 21.4% which was greater than whole wheat crackers and commercially available graham crackers that only had an activity of 1.8%. [39]

Ferric Reducing Antioxidant Power: Reciprocating to the DPPH Radical Scavenging Activity, the Ferric Reducing Antioxidant Power of the crackers was 575.71 µM Fe(II)/g that was calculated with the help of the methodology derived from *Rahim et al., 2017*[27]. Whilst comparing the values to that of whole wheat crackers, it was observed that whole wheat crackers had a FRAP value of 3.23 µmol TE/g (Trolox Equivalent) [39]

The acquired values aid in the apprehension that the microgreen incorporated crackers were rich in antioxidants. The inclusion of microgreens in the crackers elevated the antioxidant potential of the crackers. As a result of the incorporation of these antioxidant-rich compounds, crackers with a higher antioxidant potential than commercially available crackers were created.

Microbial Composition

Microbial analysis of any food product, new or old, helps identify the quantitative and qualitative presence of microorganisms in the product. On the first and third days following preparation, the crackers were microbially analyzed, and the quality of the samples was assessed.

The microbial analysis helped in the assimilation that the crackers had a minimal

microbial composition in the case of the Total Plate Count and there was no presence of Escherichia Coli and Salmonella Species.

Compared to several other crackers earlier formulated with distinguished components, the microgreen incorporated crackers had a fairly low microbial load.[36,40]

According to Indian guidelines, the total bacterial count/gram in high protein biscuits should not exceed 50,000, according to ***Nagi et al., 2012***[41]**.** These crackers are considered safe for human consumption because they are within permissible limits.

Shelf-life

The ability of a food product to maintain its sensory, proximal, physical, textural, functional, and microbiological characteristics for an extended period without alterations or modifications is referred to as shelf life as defined by ***Man, 2002***[42]**.** The shelf life of the microgreen crackers was observed in 4 different types of packages being – LDPE Zip Lock bags, Vacuum boxes, aluminium boxes, and Air-tight boxes under ambient room temperatures of about 31°C to 32°C. The vacuum box and airtight containers proved to be the best packaging methods, with no visible deterioration in any of the cracker's properties. During the shelf-life analysis, there was no visible microbial growth, but the texture of the crackers in the aluminium packaging deteriorated.

Due to the antioxidant activity of the plants as well as their phenolic activity, a study on the addition of plant extracts to biscuits concluded that the addition of plant extracts to biscuits could extend the shelf life of the biscuits. [43] As a result, it's straightforward to see how introducing microgreens to crackers could extend their shelf life.

Need For Pulse-based Products and their Importance

Recent studies have elaborated on how the need for pulse-based food products is evolving. Snacks made from pulses may be a better option to increase the nutritional value of commercially available snacks and not only for people with gluten intolerance. Due to their low price, sensory qualities, excellent nutritional profile, and environmental benefits, they constitute a key alternative for the food sector when used as ingredients in healthy snacks as well as functional foods. [44] Other studies have also elaborated on how there is a definitive need to develop more products as they are great alternatives for wheat-based products. [45] It is also suggested that there is a need for thorough research due to their functional potential in the food industries, as well as their use in non-food industries.[46]

CONCLUSION

It can thus be concluded that the formulated *Anethum graveolens L.* microgreen incorporated pulse-based gluten-free crackers are nutrient-dense with high protein, adequate dietary fibre and micronutrients, and low in fat. They also had greater sensory acceptability compared to the crackers formulated in previous studies. The crackers also exhibited great antioxidant potential and extended shelf life. Extensive research may be done with the addition of other microgreens from various families, as well as different types of pulses that can be gluten-free. Further, different antioxidant studies and assays should be carried out to estimate the true antioxidant potential of the crackers.

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Conflict of Interest

The authors present no conflict of interest.

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