Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 909 – 922 (2025)
www.ejabf.journals.ekb.eg

Potential Application of Crustacean By-Product-Derived Nano Chitosan in Gluten Free Biscuit

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ARTICLE INFO

Article History:
Received: June 6, 2025
Accepted: Aug. 30, 2025
Online: Sep. 22, 2025

Keywords:
Nanochitosan,
Spirulina,
Biscuits,
Total phenolic,
Antioxidant activity,
GC-MS
.

ABSTRACT

This study's primary goal was to assess how adding nanochitosan and spirulina to sweet and salt biscuits affects their color, physical attributes, total phenolic content, antioxidant activity, and sensory appeal to get gluten free for celiac disease. Three percent spirulina algae powder (SAP), 0.5% and 1.0% commercial chitosan (CC), nano commercial chitosan (CCN), crab chitosan (CCB), and crab nano chitosan (CCBN) were used to partially substitute quinoa flour in a variety of composite flour samples. 100g of wheat flour (WF), 100g of quinoa flour (QF), and their combinations with 3, 0.5, and 1.0% of SAP, CC, CCN, CCN, CCB, and CCBN, respectively, were used to make the cookies. Finally, biscuits made with quinoa flour and enhanced with CC, CCN, CCB, and CCBN with 3% SAP had an improved quality attributes nutritional value.

INTRODUCTION

According to **Osman *et al.* (2022)**, biscuits are a popular food among people of all ages worldwide due to their high energy content, long shelf life, and ease of digestion. There are some trends in the biscuit industry with regard to consumption and preferences: A greater focus on healthier alternatives and a growing demand for goods with better nutritional advantages. Innovation is a useful strategy for breaking into new industries and responding quickly to the demands of customers looking for more advanced products **(Lesiak *et al.*, 2018)**. When used to food enrichment, nanoparticles exhibit improved absorption. New possibilities for delivering health benefits in food are presented by nanotechnology **(Singh, 2016)**. **Razack *et al.* (2020)** used iron oxide nanoparticles as food fortifiers to increase the iron content in wheat cookies. According to **Elmotyam *et***

***al.* (2023)**, chitosan nanoparticles were shown to be more successful than regular chitosan (CS) at preventing microbial development in fisheries products. Additionally, they allowed the tested fish groups to maintain their excellent sensory scores.

Spirulina is a blue-green algae that is rich in minerals, vitamins, fibers, colors, and protein, lipids, and carbs. Biscuits are among the food items that have employed spirulina **(Setyaningsih *et al.*, 2020)**. Spirulina powder biscuits might be a good option for people who don't want to give up specific foods. They might be an alternative to already available market goods and fit in with the current movement in favor of natural meals **(Pop, 2022)**. In order to produce gluten-free cookies and snacks, **Hussein *et al.* (2025)** investigated the effects of adding spirulina algae powder to quinoa flour combinations at amounts of 3, 6, and 9% in contrast to a control comprised exclusively of QF. This study's main goal was to evaluate how chitosan nanoparticles and spirulina fortification affect the physical, color, total phenolic, antioxidant activity, and sensory qualities of sweet and salty biscuits.

MATERIALS AND METHODS

Materials

The North Cairo Flour Mills Company in Egypt provided the wheat flour (72% extraction). During the 2024-2025 season, a commercial quinoa seed sample (grown in Egypt in 2024) was purchased from the Ministry of Agriculture and was kept at 3- 4°C until it was used. The supplier of Spirulina Algae Powder (SAP) was Nourelhooda Co. in Cairo, Egypt. Commercial chitosan with a deacetylation degree of 80-95%, were purchased from Sigma Aldrich, Egypt. The crab exoskeletons (Callinectes sapidus) were obtained from a local market in Egypt. The production of CS from crab shells was conducted according to the procedure established by **Ocloo *et al.* (2011)**.

Methods

Preparation of quinoa flour

Quinoa flour was made using the **Abugoch *et al.* (2008)** technique, with certain modifications made to get rid of saponins. After being rinsed twice with cold water, whole seeds were submerged in an alkaline solution for ten to twenty minutes. They were then washed for ten minutes with a solution containing 1% citric acid. The saponins from the seed hull were eliminated when the cleaned seeds were washed with water until no foam was left behind. Seeds devoid of saponins were then dried in an oven set at 45±1°C for the whole night. The seeds were spread out thinly throughout the drying process to avoid germination and further contamination. Using a stainless-steel electric grinder and a laboratory disc mill (Quadrumat Junior flour mill, Model Type No: 279002, Brender ® OHG, Duisburg 1979, Germany), quinoa seeds were crushed into a fine powder. They were then sieved through a 60 mesh screen and were stored at 4°C until needed.

Composite flour mixture preparation

By substituting 3% spirulina algae powder (SAP), 0.5 and 1.0% commercial chitosan (CC), chitosan commercial nano (CCN), chitosan crab (CCB), and chitosan crab nano (CCBN) for quinoa flour, different composite flour samples were created. These samples were then sealed in polyethylene bags and were kept at 4˚C until they were needed.

Making salty and sweet biscuits

100 g of wheat flour (WF) as control 1, 100g of quinoa flour (QF) as control 2, and their combinations with 3, 0.5, and 1.0% of SAP, CC, CCN, CCN, CCB, and CCBN, respectively, were used to make the biscuits. The components for the biscuit recipe were as follows (Table 1): 32g of whole milk, 35g of sugar, 28g of fat, 0.93g of salt, 1.11g of baking soda, and 1g of vanilla. Making biscuits: Sugar and fat were combined until light. Milk and whole eggs were added while mixing, and the mixture was stirred for about half an hour. Salt, baking powder, and vanilla were well mixed and added to the cream mixture, which was then stirred to form a dough. After being rolled, the dough was cut into 5-cm-diameter forms. The baking process took place in a preheated oven (SHEL LAB 1370FX, USA) at 185ºC for 20 minutes. Before being analyzed, the cooled biscuit samples were stored in plastic bags.

Chemical evaluations

In accordance with **AACC (2000)** guidelines, the amounts of moisture, protein, fat, ash, and crude fiber in raw materials and biscuit samples were evaluated. Subtraction was used to calculate carbohydrates, as explained below: 100 minus (% protein + % fat + % ash + % crude fiber) equal carbohydrates.

Physical characteristics of biscuits

AACC (2000) was used to measure the biscuits' diameter (mm), thickness (mm), spread ratio, weight (gram), volume (ml), and specific volume (ml/gram). **Youssef *et al.* (2016)** used the formula Spread ratio = diameter / thickness to calculate the biscuits' spread ratio.

Determination of color

The biscuit samples' color measurements were noted. According to **Akubor and Abubakar (2020)**, the Hunter L*, a*, and b* values were acquired using a color difference meter with a Spectrocolorimeter (Tristimulus Color Machine) and the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in reflection mode.

Sensory characteristics of biscuits

Twenty semi-trained panelists from the Food Technology and Nutrition Institute team at the National Research Center in Egypt performed the sensory evaluation of the biscuit samples produced in accordance with **Linda *et al.* (1991)**. To gauge customer approval, a sensory evaluation was carried out. For sensory evaluation, a numerical hedonic scale ranging from 1 to 20 was used, where 1 represents the least liked and 20 represents the most loved.

Volatile compounds

The analysis was performed in the National Research Center, Giza, Egypt using an Agilent 8890 GC System, coupled to a mass spectrometer (Agilent 5977B GC/MSD) according to **Centonze *et al.* (2019)**.

Table 1. Formulation of gluten-free sweet and salted biscuit

Ingredients (gram)	Control (1)	Control (2)	1	2	3	4	5	6	7	8
Gluten-free sweet biscuit										
WF	100	-	-	-	-	-	-	-	-	-
QF	-	100	96.5	96.0	96.5	96	96.5	96.0	96.5	96.0
SAP	-	-	3	3	3	3	3	3	3	3
CC	-	-	0.5	1.0	-	-	-	-	-	-
CCN	-	-	-	-	0.5	1.0	-	-	-	-
CCB	-	-	-	-	-	-	0.5	1.0	-	-
CCBN	-	-	-	-	-	-	-	-	0.5	1.0
Sugar	35	35	35	35	35	35	35	35	35	35
Butter	28	28	28	28	28	28	28	28	28	28
Whole milk	32	32	32	32	32	32	32	32	32	32
Baking powder	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
vanilla	1	1	1	1	1	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Gluten-free salted biscuit										
WF	100	-	-	-	-	-	-	-	-	-
QF	-	100	96.5	96.0	96.5	96.0	96.5	96.0	96.5	96.0
SAP	-	-	3	3	3	3	3	3	3	3
CC	-	-	0.5	1.0	-	-	-	-	-	-
CCN	-	-	-	-	0.5	1.0	-	-	-	-
CCB	-	-	-	-	-	-	0.5	1.0	-	-
CCBN	-	-	-	-	-	-	-	-	0.5	1.0
Butter	28	28	28	28	28	28	28	28	28	28
Whole milk	32	32	32	32	32	32	32	32	32	32
Baking powder	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
vanilla	1	1	1	1	1	1	1	1	1	1
Salt	5	5	5	5	5	5	5	5	5	5

Where: WF: Wheat flour; QF:Quinoa flour; SAP: Spirulina Algae Powder; CC: Chitosan commercial; CCN: Chitosan commercial nano; CCB; Chitosan crab; CCBN: Chitosan crab nano.

Statistical analyses

The standard deviation (SD) was calculated using Excel 2010 software. Statistical evaluation was performed utilizing the Co State software via a one-way analysis of variance (ANOVA). The statistical evaluation of the results acquired was conducted in triplicate **(Silva *et al.*, 2009)**.

RESULTS and DISCUSSION

Proximate composition of raw materials

To make gluten-free biscuits, quinoa flour (QF), wheat flour (WF), and spirulina algae powder (SAP) were used. Together with wheat flour, gluten-free flour samples were subjected to chemical analysis, as indicated in Table (2).

Table 2. Approximate analysis (% on dry weight basis) of used raw materials, sweet and salted biscuit samples

Samples	Moisture	Ash	Protein	Fat	Fiber	CHO
Raw materials						
Wheat flour	13.43±0.45	0.58±0.01	11.56±0.28	1.14±0.06	0.51±0.05	86.22±0.41
Quinoa flour	9.64±0.53	2.70±0.09	18.95±0.63	5.22±0.26	5.41±0.11	67.72±0.92
Spirulina powder	7.92±0.20	11.47±0.14	58.49±0.37	5.75±0.09	4.77±0.08	19.52±0.50
Sweet biscuit samples						
Control wheat	3.87[e]	1.17[c]	7.65[c]	18.07[b]	0.37[d]	72.74[b]
Control quinoa	4.65[d]	2.41[b]	11.57[b]	20.19[a]	3.31[c]	62.52[a]
1	5.09[c]	2.57[a]	12.21[a]	20.15[a]	3.58[b]	61.49[a]
2	5.11[c]	2.52[a]	12.15[a]	20.11[a]	3.79[a]	61.43[a]
3	6.43[ab]	2.55[a]	12.21[a]	20.17[a]	3.55[b]	61.52[a]
4	6.87[a]	2.54[a]	12.19[a]	20.16[a]	3.75[a]	61.39[a]
5	5.32[c]	2.59[a]	12.15[a]	20.13[a]	3.59[b]	61.54[a]
6	5.21[c]	2.54[a]	12.17[a]	20.18[a]	3.73[a]	61.38[a]
7	6.15[b]	2.58[a]	12.21[a]	20.15[a]	3.58[b]	61.48[a]
8	6.85[a]	2.55[a]	12.16[a]	20.14[a]	3.77[a]	61.38[a]
LSD at 0.05	0.408	0.112	0.437	0.958	0.129	1.314
Salted biscuit samples						
Control wheat	3.01[f]	5.01[d]	9.80[c]	23.16[b]	0.48[d]	61.55[a]
Control quinoa	3.96[e]	6.44[c]	14.72[b]	25.68[a]	4.21[c]	48.95[b]
1	4.22[d]	6.60[a]	15.53[a]	25.62[a]	4.45[b]	47.80[b]
2	4.67[c]	6.57[b]	15.45[a]	25.60[a]	4.52[a]	47.86[b]
3	5.13[b]	6.64[a]	15.50[a]	25.59[a]	4.43[b]	47.84[b]
4	5.29[b]	6.60[a]	15.43[a]	25.60[a]	4.55[a]	47.82[b]
5	4.75[c]	6.63[a]	15.49[a]	25.65[a]	4.41[b]	47.82[b]
6	4.88[c]	6.59[a]	15.46[a]	25.59[a]	4.59[a]	47.77[b]
7	5.60[a]	6.62[a]	15.55[a]	25.63[a]	4.45[b]	47.75[b]
8	5.82[a]	6.56[b]	15.47[a]	25.58[a]	4.57[a]	47.82[b]
LSD at 0.05	0.295	0.09	0.563	1.469	0.061	1.958

According to the data, all of the flour samples had moisture contents ranging from 7.92 to 13.43 percent. Compared to WF (protein 11.56% & fat 1.14%), SAP was distinguished by having a greater protein (58.49%) and fat (5.75%) concentration. SAP and QF had the lowest levels of total carbohydrates, falling to 19.52 and 67.72%, respectively. These SAP chemical composition results are consistent with earlier findings demonstrated by **Sahin (2020)**. The WF chemical composition results are consistent with earlier findings published by **Hussein *et al.* (2018, 2019)**. WF 1.14% had a lower fat percentage, but QF (5.22%) and SAP (5.75%) had greater fat contents. Additionally, QF and SAP have larger fiber contents than WF. Additionally, a number of researchers assessed the chemical makeup of **Yegrem (2021)**, a previously chosen free gluten flour.

Chemical composition of biscuit

Table (2) displays the chemical composition of the control biscuits (WF or QF) and the ones enhanced with SAP and CC or CCB. The biscuit samples that were based on CC and CCB had significantly greater moisture levels than the control biscuit samples. Displaying the greatest levels of moisture (6.87 and 6.85%, respectively). On the other hand, the 100% WF control samples had a moisture level of 3.87%, but the 100% QF control biscuit had a moisture content of 4.65%. The biscuits' ash content varied from 1.17 to 2.59%. On the other hand, compared to the control biscuits (100% WF or 100% QF), the protein content of the biscuits containing SAP and CC or CCB was significantly greater ($P<0.05$). In general, the SAP-enhanced cookie samples had more protein than the control samples. However, when comparing the fat content of most fortified biscuits to the biscuit control (100% QF), no discernible variations were seen. A highly significant difference in fiber levels between the control biscuits and those supplemented with CC or CCB was found by the statistical analysis. Perhaps as a result of the initially low carbohydrate levels in the raw materials employed in the combinations, the carbohydrate content of the biscuits gradually decreased in all samples as the replacement ratio of CC or CCB increased. However, these declines were not significant. When compared to control biscuits, all of the biscuits made in this study may be categorized as bakery goods with higher quantities of ash and fiber. These results are consistent with those of **Wang *et al.* (2016)**. The chemical composition of salt biscuits boosted with different concentrations of chitosan (CC and CCB) and 3% SAP is shown in Table (2). Regarding moisture, protein, ash, fat, fiber, and carbs, the same results were seen for sweet biscuits.

Baking quality of biscuits

In contrast to control samples (100% WF or 100% QF), the weight (g), volume (cm3), specific volume (v/w), diameter (cm), thickness (cm), and spread ratio (%) of biscuit samples created by substituting different quantities of chitosan (CC or CCB) together with 3% SAP are displayed in Table (3). As the substitution % rose, the CC and CCB biscuits' diameter somewhat decreased, but not significantly. The 1% CCB cookies had the smallest diameter, 5.08 cm, whereas the control samples had the biggest, 5.63 cm. According to **Miller and Hoseney (1997)**, biscuits of superior quality should have a high spread ratio, which is the ratio of diameter to thickness and is a measure of biscuit quality. The findings showed that, as compared to control biscuit samples, the spread ratio decreased with the addition of CC and CCB, with no discernible increase. When compared to the control sample, adding chitosan to the biscuit-making process generally did not produce a discernible change in the spread ratio. Despite the fact that the generated biscuits' thickness, specific volume, and weight varied, significantly.

Table 3. Baking quality of sweet and salted biscuit samples

Samples	Weight (g)	Volume (cm3)	Specific volume (cm3/g)	Thickness (cm)	Diameter (cm)	Spread ratio (%)
Sweet biscuit samples						
Control wheat	10.83^{d}	14.98^{f}	1.38^{a}	0.62^{cd}	5.27^{bc}	8.50^{bc}
Control quinoa	12.48^{bc}	15.23^{ef}	1.22^{c}	0.55^{e}	5.63^{a}	10.24^{a}
1	12.47^{c}	16.33^{cd}	1.31^{ab}	0.60^{d}	5.37^{b}	9.02^{b}
2	12.76^{bc}	16.44^{cd}	1.29^{bc}	0.61^{cd}	5.23^{cd}	8.57^{bc}
3	12.99^{abc}	17.15^{abc}	1.32^{ab}	0.65^{abc}	5.22^{cd}	8.09^{cd}
4	13.28^{abc}	17.40^{ab}	1.31^{ab}	0.67^{ab}	5.12^{de}	7.70^{de}
5	12.82^{bc}	16.82^{bcd}	1.31^{ab}	0.67^{ab}	5.12^{de}	7.64^{de}
6	13.34^{ab}	16.93^{abc}	1.27^{bc}	0.69^{a}	5.08^{e}	7.41^{e}
7	12.92^{abc}	15.99^{de}	1.24^{bc}	0.62^{cd}	5.24^{bcd}	8.45^{bc}
8	13.73^{a}	17.74^{a}	1.29^{bc}	0.64^{bcd}	5.19^{cde}	8.17^{cd}
LSD at 0.05	0.866	0.911	0.081	0.044	0.138	0.598
Salted biscuit samples						
Control wheat	9.49^{d}	15.03^{e}	1.59^{a}	0.61^{bc}	4.82^{ab}	7.97^{b}
Control quinoa	11.12^{c}	15.38^{e}	1.38^{b}	0.54^{d}	5.09^{a}	9.50^{a}
1	11.15^{c}	16.49^{cd}	1.48^{ab}	0.59^{cd}	4.82^{abc}	8.23^{b}
2	11.43^{bc}	16.74^{cd}	1.46^{b}	0.61^{bc}	4.71^{bc}	7.79^{b}
3	11.67^{abc}	17.10^{bc}	1.47^{b}	0.63^{abc}	4.72^{bc}	7.56^{bcd}
4	12.02^{ab}	17.60^{ab}	1.46^{b}	0.65^{ab}	4.57^{bc}	7.03^{cd}
5	11.50^{bc}	16.86^{c}	1.47^{b}	0.66^{ab}	4.52^{c}	6.90^{d}
6	12.01^{ab}	16.99^{bc}	1.41^{b}	0.67^{a}	4.63^{bc}	6.91^{d}
7	11.64^{abc}	16.09^{d}	1.38^{b}	0.61^{bc}	4.74^{bc}	7.83^{b}
8	12.40^{a}	17.77^{a}	1.43^{b}	0.62^{abc}	4.70^{bc}	7.64^{bc}
LSD at 0.05	0.814	0.659	0.107	0.061	0.296	0.733

Sensory evaluation of biscuits

Table (4) shows the effects of adding CC and CCB to biscuits at different concentrations on the biscuits' color, flavor, scent, crispness, appearance, and general acceptance. The results showed that in every sensory attribute analyzed, the control sample performed noticeably better. The control sample and biscuits enriched with CC and CCB combined with 3% SAP had superior flavor, fragrance, color, crunchiness, appearance, and overall preference (probability level P>0.05) compared to the control biscuit (100% QF). When it came to flavor, scent, color, crispness, appearance, and overall attractiveness, biscuits made entirely with WF scored highest. It was shown that adding more CC and CCB decreased the qualities of taste, fragrance, color, crunchiness, appearance, and overall likeability. It was decided that biscuits enhanced with different concentrations of CC and CCB were acceptable. According to the findings of the sensory test, QF biscuits can be fortified with 1% CC and 1% CCB.

Table 4. Sensory evaluation of sweet and salt biscuit samples

Samples	Color (20)	Taste (20)	Crispness (20)	Odor (20)	Appearance (20)	Overall-acceptability (100)
Sweet biscuit						
Control wheat	18.65^{a}	18.78^{a}	18.84^{a}	19.43^{a}	18.15^{a}	93.84^{a}
Control quinoa	15.50^{b}	15.29^{e}	17.46^{bc}	18.23^{b}	15.05^{b}	81.52^{b}
1	15.95^{b}	16.58^{bc}	17.69^{b}	17.90^{bc}	15.45^{b}	83.57^{b}
2	15.75^{b}	15.65^{de}	16.16^{d}	17.55^{bc}	15.25^{b}	80.36^{b}
3	15.85^{b}	16.60^{bc}	16.95^{c}	17.74^{bc}	15.20^{b}	82.34^{b}
4	16.02^{b}	15.80^{bcde}	15.88^{d}	17.38^{c}	15.55^{b}	80.63^{b}
5	15.95^{b}	16.35^{bcd}	17.07^{bc}	17.97^{bc}	15.35^{b}	82.69^{b}
6	15.90^{b}	15.83^{bcde}	15.82^{d}	17.26^{c}	15.35^{b}	80.15^{b}
7	15.90^{b}	16.65^{b}	16.98^{c}	$17.86b^{c}$	15.30^{b}	82.69^{b}
8	16.00^{b}	15.70^{cde}	16.02^{d}	17.42^{bc}	15.48^{b}	80.61^{b}
LSD at 0.05	1.671	0.908	0.641	0.828	1.320	3.959
Salted biscuit						
Control wheat	17.35^{abc}	16.85^{e}	16.10^{d}	18.20^{a}	17.00^{bcd}	85.50^{cd}
Control quinoa	17.75^{abc}	16.84^{e}	17.25^{bc}	17.85^{ab}	17.37^{abc}	87.05^{abcd}
1	17.10^{c}	16.95^{de}	17.17^{c}	17.45^{bc}	16.74^{d}	85.41^{d}
2	17.30^{abc}	17.15^{cde}	17.39^{bc}	17.30^{bc}	16.98^{bcd}	86.12^{bcd}
3	17.15^{bc}	17.58^{abc}	17.37^{bc}	17.10^{c}	16.83^{cd}	86.02^{bcd}
4	17.60^{abc}	17.90^{ab}	17.90^{ab}	17.05^{c}	17.21^{abcd}	87.66^{abc}
5	17.20^{bc}	17.05^{cde}	17.15^{c}	17.40^{bc}	16.80^{cd}	85.60^{cd}
6	17.71^{abc}	17.10^{cde}	17.25^{bc}	17.20^{bc}	17.36^{abc}	86.62^{bcd}
7	17.85^{ab}	17.45^{bcd}	17.80^{abc}	17.35^{bc}	17.45^{ab}	87.90^{ab}
8	17.95^{a}	18.10^{a}	18.15^{a}	17.25^{bc}	17.63^{a}	89.08^{a}
LSD at 0.05	0.744	0.586	0.666	0.691	0.618	2.164

Total phenolic and antioxidant activity of biscuits

It has been suggested that phenol and flavonoids, two important classes of non-essential dietary components, might improve human health **(Celestine *et al*., 2013;**

Upadhyaya *et al.*, 2017). Table (5) gives the bioactive compound results for the sweet and salt biscuits under investigation. The amounts of total phenols in salt and sweet biscuits were measured in milligrams of gallic acid equivalent per gram (mg GAE/g). The sweet biscuit with 1% CC showed an antioxidant activity of 83.46 (ug TE/g) and a total phenol content of 222.94mg/ g. By contrast, the salt biscuits containing 0.5% CCB showed an antioxidant activity of 73.57 (ug TE/g) and a total phenol content of 182.22mg/ g. These findings align with those published by **Al-Juhaimi (2014)**.

Table 5. Total phenolic and antioxidant activity of sweet and salt biscuits

Samples	Total phenols (ug GAE/g)		DPPH (ug TE/g)	
	Sweet biscuits	Salt biscuits	Sweet	Salt biscuits
Control wheat	127.80	127.80	169.01	169.01
Control quinoa	25.56	25.56	73.96	73.96
1	42.57	132.56	73.90	74.24
2	222.94	38.71	83.46	74.68
3	34.03	177.06	73.85	74.98
4	123.54	81.15	73.96	74.15
5	90.69	182.22	74.98	73.57
6	29.94	82.56	74.25	75.44
7	139.02	139.79	75.43	73.54
8	67.78	47.19	73.54	74.49

Volatile compounds of biscuits

The volatile oils isolated from biscuits were characterized using GC-MS analysis (Table 6). 99.65% of the total oils were found to consist of the 63 components that were found. Palmitic acid (26.4%), D-limonene (9.67%), 2,6,11-trimethyldodecane (7.84%), palmitoleic acid (5.73%), β-pinene (2.35%), estragole (5.04%), linalyl acetate (4.6%), vanillin (3.1%), p-cymene (2.33%), γ-terpinene (2.11%), ethylene acetate (1.6%), and undecane (1.17%) were the main ingredients in the CCBN biscuit. The same species was studied in Egypt, Iran, Spain, Mexico, and the USA by **Ayala *et al.* (2017)**, **Xu *et al.* (2017)**, **Golmohammadi *et al.* (2018)** and **Ibrahim and El-Sawi (2019)**, with similar results. The variations in the primary constituents of the essential oil can be ascribed to diverse cultivation conditions, geographical locations, seasonal fluctuations, and extraction techniques **(Ghazanfari *et al.*, 2020)**

Table 6. Volatile compounds of biscuits

Peak	RT	Compounds	Control	1	2	3	4	5	6	7	8
1	2.133	Ethyl Acetate	0.46	nd	nd	nd	nd	2.83	nd	3.5	1.6
2	2.479	Formic acid	nd	nd	nd	nd	nd	nd	2.65	5.33	nd
3	2.675	Acetic acid	nd	nd	nd	nd	nd	nd	nd	3.73	nd
4	2.746	-2(Aminooxy)propionic acid	nd	nd	nd	nd	nd	nd	1.68	1.94	nd
5	3.174	Acethydrazide	nd	nd	nd	nd	nd	nd	nd	1.93	nd
6	5.832	Undecane	nd	nd	nd	nd	nd	nd	nd	nd	1.17
7	6.076	Furfuralcohol	0.49	nd	nd	nd	nd	6.31	3.9	8.02	nd
8	7.033	Styrene	0.53	0.61	0.52	nd	0.7	nd	nd	nd	nd
9	7.812	Methyl Nhydroxybenzenecarboxim-idoate	nd	nd	nd	nd	nd	1.48	3.35	1.52	1.15
10	8.359	-3Carene	1.53	2.91	2.3	1.1	1.4	nd	nd	nd	nd
11	9.167	.(E)-2-Heptenal	nd	nd	0.67	nd	nd	nd	nd	nd	nd
12	9.275	Benzaldehyde	0.59	0.65	0.55	nd	0.63	nd	nd	nd	nd
13	9.792	-(-).β-Pinene	0.8	1.85	1.93	0.59	1.11	nd	nd	nd	nd
14	10.214	-6Methyl-5-heptene-2-one	0.6	0.66	1	nd	0.95	nd	1.68	nd	nd
15	10.327	β-Myrcene	3.36	3.94	5.31	2.43	4.25	nd	nd	1.36	2.33
16	11.498	p-Cymene	4.09	4.95	5.42	3.85	5.25	nd	1.53	2.14	2.49
17	11.671	D-Limonene	13.15	16.2	17.9	12.17	16.8	2.89	3.49	8.24	9.67
18	11.754	Eucalyptol	2.27	4.03	5.36	1.54	4.46	2.38	5.78	nd	nd
19	12.729	γ-Terpinene	3.49	4.46	4.6	3.75	4.44	nd	nd	1.83	2.11
20	13.817	Fenchone	nd	nd	0.37	nd	nd	nd	1.8	nd	nd
21	14.073	Cyclopentanol	nd	nd	nd	nd	nd	1.78	3.68	4.84	nd
22	14.239	Linalool	0.73	0.82	1.14	0.51	1.31	nd	1.57	2.84	nd
23	14.709	Maltol	0.39	nd	nd	nd	nd	nd	nd	nd	nd
24	15.827	-2-(+)Bornanone	0.78	1.21	2.01	nd	1.65	1.65	3.35	nd	nd
25	16.172	l-Menthone	3.3	4.14	5.42	3.06	5.85	2.81	4.85	1.93	nd
26	16.547	cis-p-Menthan-3-one	0.99	1.24	1.95	0.86	1.93	2.51	2.81	2.95	nd
27	16.885	Levomenthol	0.49	0.83	0.84	0.63	1.16	nd	nd	nd	nd
28	17.813	Estragole	17.72	22.73	17.41	23.78	23.64	7.33	11.76	7.13	5.04
29	17.998	(E)-3-Decenol	nd	nd	nd	nd	nd	1.79	nd	nd	nd
30	18.58	-5Hydroxymethyldihydrofuran-2-one	nd	nd	nd	nd	nd	1.96	nd	nd	nd
31	18.61	γ-n-Heptylbutyrolactone	nd	nd	nd	nd	nd	2.31	nd	nd	nd
32	18.955	-5Hydroxymethylfurfural	nd	nd	nd	nd	nd	9.68	nd	nd	nd
33	19.222	Cuminaldehyde	5.49	7	5.57	7.73	6.83	5.21	6.77	3.45	1.2
34	19.371	-(-)Carvone	3.65	4.99	3.76	5.51	4.96	5.22	6.85	4.03	1.33
35	19.746	Linalyl acetate	0.48	0.64	0.78	0.75	0.65	nd	nd	nd	4.6
36	20.263	Citral	1.34	1.82	1.5	2.5	1.43	1.63	2.67	1.36	nd
37	20.786	Anethole	4.81	9.18	7.25	15.34	5.8	1.52	2.26	1.4	nd
38	20.953	γ-Terpinen-7-al	0.56	0.87	0.52	0.89	0.91	nd	nd	nd	nd
39	21.036	-2Undecanone	0.38	0.47	0.56	nd	nd	nd	nd	nd	nd
40	21.744	-2Acetyl-4-methylphenol	nd	nd	nd	nd	nd	nd	nd	1.35	2.49
41	21.946	Eicosyl isopropyl ether	nd	nd	nd	nd	nd	nd	nd	nd	1.36
42	22.689	Isosorbide Dinitrate	nd	nd	nd	nd	nd	1.93	nd	1.45	nd
43	22.9	α-Terpinyl acetate	nd	0.53	0.53	0.67	0.52	nd	nd	nd	nd

44	24.544	Vanillin	3.72	3.27	4.36	8.51	1.97	4.54	8.22	11.6	3.1
45	27.345	-2,6,11Trimethyldodecane	nd	nd	nd	nd	nd	nd	nd	nd	7.84
46	27.636	-2,6,10,15Tetramethylheptadecane	nd	nd	nd	nd	nd	nd	nd	nd	1.51
47	28.344	Ethyl 4-ethoxybenzoate	nd	nd	nd	nd	nd	1.66	nd	nd	nd
48	28.724	-4,6Dimethyldodecane	nd	nd	nd	nd	nd	nd	nd	nd	5.23
49	31.959	-4(3-Hydroxy-2-methoxyphenyl)-2-butanone	nd	nd	nd	nd	nd	12.75	7.69	3.95	1.14
50	33.308	Heptadecane	nd	nd	0.45	1.19	nd	nd	nd	nd	1.22
51	35.057	Myristic acid	0.88	nd	nd	nd	nd	nd	nd	1.34	nd
52	36.674	Isopropyl myristate	nd	nd	nd	0.81	nd	nd	nd	nd	nd
53	36.84	Bufalin	0.38	nd	nd	0.65	nd	nd	nd	nd	nd
54	37.601	Pentadecanoic acid	0.42	nd	nd	nd	nd	nd	nd	nd	nd
55	39.593	Palmitoleic acid	1.23	nd	nd	nd	nd	nd	nd	1.34	5.73
56	40.188	Palmitic acid	6.21	nd	nd	0.59	nd	10.44	6.21	8.15	26.4
57	41.544	Isopropyl palmitate	nd	nd	nd	0.61	nd	nd	nd	nd	1.72
58	43.56	-2,5Dibutylfuran	nd	nd	nd	nd	nd	3.72	nd	nd	nd
59	44.148	trans-13-Octadecenoic acid	0.84	nd	nd	nd	nd	1.87	2.29	nd	6.97
60	44.677	Stearic acid	0.51	nd	nd	nd	nd	1.05	nd	nd	2.6
61	48.596	Adipic acid, bis(2-ethylhexyl) ester	10.98	nd	nd	nd	nd	nd	nd	nd	nd
62	50.267	Phthalic acid, di(2-propylpentyl) ester	1.27	nd	nd	nd	nd	nd	nd	nd	nd
63	52.187	-1,4Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	1.11	nd	nd	nd	nd	nd	nd	nd	nd

nd: not detected.

CONCLUSION

In conclusion, the nutritional content and qualitative features of quinoa flour biscuits fortified with CC, CCN, CCB, and CCBN with 3% SAP were enhanced. 1% CCBN-fortified biscuits were the most susceptible overall, followed by 1% CCB-fortified biscuits. Additionally, in the biscuit sector, quinoa flour might be substituted with 3.0% SAF and 1% CCBN, which would have positive effects and higher nutritional value for celiac disease.

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