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Enhancement of Nutritional Quality of Pretzels Using Sprouted Garden Cress Seeds

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Abstract

The aim of this paper is to enhance the nutritional quality of pretzels using sprouted garden cress seeds. Sprouting was performed on garden cress seeds at 3 and 6 days to select the best sprouting conditions. Chemical composition, total phenols, flavonoids, and DPPH were measured on sprouted and un-sprouted garden cress. Sprouted and un-sprouted garden cress seeds were substituted at 0, 5, 10, and 15% levels with wheat flour in pretzels formula. Chemical analysis, sensory properties, and protein quality were performed on pretzels to assess their nutritional quality. After 3 and 6 days, sprouting increased ($P \leq 0.05$) ash (9.89, 5.88%), fiber (10.20, 9.22%), and protein (24.71, 25.92%). Sensory scores showed no ($P \leq 0.05$) differences between pretzels with sprouted and un-sprouted garden cress at 5 and 10% for all attributes. Pretzels with 10% sprouted garden cress was the highest ($P \leq 0.05$) of all tested minerals. *In vitro* protein digestibility (78.46%), total essential amino acids (46.3%), and total non-essential amino acids (71.8 %) were increased in pretzels with 10% sprouted garden cress compared to un-sprouted seeds. Sprouted garden cress could be used to improve the nutritional quality of pretzels at 10% level of substitution with wheat flour with highly acceptability. This study leads to a valuable addition and improvement of the consumer's diet, especially with the limited exploration of the potential of sprouted garden cress seed in a popular bakery snack like pretzels.
Keywords: Garden Cress; Sprouting; Pretzels; Chemical Analyses; Sensory Evaluation.

1. Introduction

Pretzels are a very tasty, low calorie; nutritious, baked and cooked products usually made from wheat and are prevalent snack foods in many developed countries. Pretzels manufacturing and consumption are rising quickly as a result of customer demand for alternatives to fried snack foods. To suit the growing customer demand, pretzel production is currently totally automated in developed countries [1].

The development of new products is a strategic area of the food industry. Consumers must treat food requirements with two main characteristics; it is expected to deal with the traditional nutritional properties of foods and the additional health benefits from their regular intake. These distinct foods are called nutritious foods. Regarding changes in food consumption habits and stressful lifestyles, a healthy digestive system is an important issue that also leads to an increase in overall quality of life [2].

Wheat flour is an excellent source of energy but limits in vitamins such as vitamin C and beta-carotene, minerals, especially iron, and some essential amino acids. Otherwise, these nutrients are widely available in various types of fruits, vegetables, grains and other seeds. Thus, it was important to supplement the bakery products made of wheat flour with other sources which rich in nutrients to compensate for the deficiency in wheat flour [3,4].

Garden cress (*Lepidium sativum* L.) is an edible plant and a member of the *Cruciferae* (*Brassicaceae*) family. It is an erect annual plant grown as a culinary vegetable in many countries and is native to Egypt [5]. Cress seeds have the potential to be used as a nutritional and therapeutic agent. It is a fast-growing, perennial edible plant, botanically related to mustard and water cress. It is cool season herb that is native to Egypt and West Asia but is widely cultivated in hot temperate climates throughout the world for various culinary and medicinal uses [6].

Garden cress seeds (GCS) are categorized under oil seeds and are enriched with macro and micronutrients. The seeds of the garden cress possess good levels of protein (21-25%), fat (23- 27%),

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carbohydrate (30-34%), dietary fiber (30%), phosphorus (723 mg/100 g), magnesium (430 mg/100 g), calcium (296-377mg/100 g), iron (76-100 mg/100 g), zinc (5 mg/100 g) and thus an important nutraceutical seed for nutrient enrichment [7,8]. Protein of GCS has a good quality, containing essential amino acids, including lysine and phenylalanine. The major fatty acids are oleic (30.6%), linolenic acid (30.2%), with low amount of erucic acid (3.9%). GCS oil has balanced amount of monounsaturated fatty acids (37.6%) and polyunsaturated fatty acids (46.8%), also contains natural antioxidants. Its high nutritional value, therapeutic properties and low price facilitate its availability in meals and enhance the nutritional quality of meals.

Many supplementation and compensation studies have been conducted on garden cress seeds (protein and fats rich) and its products which really had good results, where garden cress extract or powder can be added in fruits and vegetable juices which are rich in vitamins and minerals but are poor in proteins and fats [9,10].

Despite the nutraceutical potentials of cress seeds, however, it did not receive great attention and remained underutilized. Garden cress acceptability for use in food products is significantly decreased by its mild bitterness and astringency. Bitterness of the seeds due to high polyphenol content is one of the factors which significantly decrease its acceptability and bioavailability of minerals [11,12]. With this backdrop, cress seeds were subjected to different processing treatments such as roasting, popping and germination [13-15]. Several seeds can be eaten after germination. The germination of seeds is very easy, cheap, and has been known for ages, especially in our Eastern culture, and it has been generally stated that seed sprouts have more nutritional value, phenol content, and antioxidant activity than dry seeds [16,17].

Accordingly, this study aims to enhance the nutritional quality of pretzels using sprouted garden cress seeds (0, 5, 10 and 15 %). Sprouting was performed in garden cress seeds at 3 and 6 days to select the best sprouting conditions. Chemical composition, antioxidant properties, sensory scores and protein quality were performed to assess the quality of the final products.

2. Materials and Methods

2.1. Materials

Garden cress seeds were obtained from local market, Cairo, Egypt. Wheat flour of 72% extraction rate was obtained from South Cairo Mills Company, Giza, Egypt. Pretzels ingredients (oil, sugar, yeast, salt and milk powder) were purchased from local market, Cairo, Egypt. All chemicals were of analytical grade and obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Sprouting of garden cress seeds

The garden cress seeds (GCS) were sorted and cleaned to remove impurities. The seeds were washed and drained. The seeds were divided into two parts, the first part was germinated in the laboratory. The seeds were spread on muslin cloth and kept at ambient temperature (18-25 °C) / 6 days. The second part germinated in the soil for 6 days. After germination, the sprouts with rootlets were harvested after 3 and 6 days of growth and then dried at 40±5°C. Later, sprouted seeds were milled to produce a fine-textured powder.

2.2.2. Chemical composition of GCS/sprouted seeds (SGC)

The chemical constituents of GCS and SGC, including moisture, ash, fat, fiber, and protein, were determined in triplicate according to the AOAC [18]. The difference was used to calculate the total carbohydrate content.

Considering the findings of analysing the chemical composition of germinated garden cress seeds under laboratory and soil conditions, the best germination conditions that improved the chemical composition of the resulting germinated seeds were determined in order to study their content of phenolic substances as well as their antioxidant activity for their application in the manufacture of pretzels.

2.2.3. Preparation of GCS/SGC extracts

The GCS/ SGC extracts were prepared according to Abdel-Aty *et al.* [6]. Garden cress seeds or sprouted seeds (2 g) were dried in oven at 50 °C for 4hrs. and extracted by shaking at 150 rpm for 24hrs with 20 ml methanol (80%) and filtered through filter paper Whatman No. 1. the filtrate designated as methanol extract.

2.2.4. Determination of total phenolics and flavonoids of GCS and SGC extracts

Total phenolics concentration was measured according to Velioglu *et al.* [19]. Fifty microliters of the methanol extract were mixed with 100µl Folin-Ciocalteu reagents, 850µl of distilled water and allowed to stand for 5 min at room temperature. A 500 µl of 20% sodium carbonate was added and allowed to react for 30 min. Absorbance was measured spectrophotometrically at a wavelength of 765nm (HITACI, U-1900). Total phenols were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of gallic acid. The results are expressed as mg gallic acid equivalent (GAE)/g DW.

Total flavonoids concentration was determined using a modified colorimetric method described by Zhishen *et al.* [20] and used catechin as a standard. Methanol extract or standard solution (250 µl) was mixed with distilled water (1.25 ml) and 5% $NaNO_2$ solution (75 µl). After standing for 6 min, the mixture was combined with 10% $AlCl_3$ solution (150 µl). One M NaOH (0.5 ml) and distilled water (275 µl) was added to the mixture 5 min later. The absorbance of the mixture was measured at 415 nm with UV-Vis spectrophotometer (HITACI, U-1900). Total flavonoids were quantified from a calibration curve obtained by measuring the absorbance of known concentrations of catechin. The results expressed as mg catechin equivalent (CE)/g DW.

2.2.5. Antioxidant assays of GCS and SGC extracts

Free radical scavenging activity of methanol extract was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method of Ao *et al.* [21]. A 0.1 ml methanol extract was mixed with 0.9 ml of freshly prepared DPPH methanol solution (0.1 mM). An equal amount of methanol was used as a control. After incubation for 30 min at room temperature in the dark, the optical density (OD) was measured at 517 nm using a spectrophotometer (HITACI, U-1900). The scavenging activity of extracts was compared with that of a synthetic antioxidant (BHT) at 4 concentrations (50, 100, 150 & 200 ppm). Activity of scavenging (%) was calculated using the following formula:

$$\text{DPPH radical scavenging \%} = [(OD_{control} - OD_{sample})/OD_{control}] \times 100 \quad (1)$$

2.2.6. Pretzels Preparation

Pretzel samples were prepared according to the method described by El-Gohery [22] using wheat flour 72% (control), garden cress seed powder (GCS) and sprouted garden cress powder (SGC) at different replacement levels which are recorded in Table (1). The dry ingredients were mixed for 1 min in a lab mixer, then water was added as required for each sample, and the mixture was further mixed for 4 min to form the dough. The resulting dough could rest for 20 min before shaping. The shaped pretzel was baked at 180 C° for 10 min, then sprayed with a caustic solution (1% sodium hydroxide). Finally, the produced pretzels were baked at 93 C° /30 min.

2.2.7. Sensory evaluation of Pretzels

Sensory evaluation was carried out on pretzels according to Mc Williams [23]. Members from Food Technology Research Institute, Agricultural Research Center scored pretzels for appearance, colour, odour, taste, mouth feel, crispness, and overall acceptability (OAA) on a 9 hedonic scale from one (dislike extremely) to nine (like extremely).

2.2.8. Analytical Methods

Moisture, protein, ash, crude fiber and fat of the produced pretzels were determined according to the AOAC [18]. Total carbohydrates were calculated by difference. Minerals including; Magnesium (Mg), sodium (Na), potassium (K), calcium (Ca), iron (Fe), phosphorus (P) and zinc (Zn) were determined by using the atomic absorption spectrophotometer (Perkin-Elmer model 3300) as described by Kirleis *et al.* [24].

2.2.9. Determination of protein digestibility (in vitro)

Protein digestibility (in vitro) of each pretzels sample was determined based on the method of Schlemmer *et al.* [25]. Briefly, a one-gram sample was placed into a 100 ml Erlenmeyer flask containing 15mL of pepsin solution (1.5 mg pepsin in HCl 0.1 M), then the flask was incubated at 37°C for 3 hrs. The suspension was neutralized with NaOH (7.5 ml, 0.2M), then treated with 7.5 ml of pancreatin solution (4mg in 0.2 M phosphate buffer, pH 8.0).

One ml of toluene was added to prevent microbial growth, and the mixture was gently shaken and incubated for 24 hrs at 37°C. After incubation, the sample was treated with 10ml of 10% TCA to remove undigested protein and larger peptides and centrifuged at 5000 xg for 20 min at 25±5° C. Protein in the supernatant was determined using the Kjeldahl method AOAC [18]. Protein digestibility (%) was calculated using the following formula:

$$Protein\ digestibility(\%) = Ncontent\ in\ supernatant - N\ content / N\ content\ in\ sample \times 100 \quad (2)$$

2.2.10. Amino acids content and protein quality

The amino acid contents of pretzel samples were determined using the Automatic Amino Acid Analyzer (BIOCHROM 30, serial 103274), according to the method outlined in AOAC [18]. The chemical score of essential amino acids (EAA) was relatively calculated according to [26] using the following:

$$Chemical\ score\ (\%) = essential\ amino\ acid\ of\ crude\ protein / essential\ amino\ acid\ of\ (FAO/WHO)\ X\ 100 \quad (3)$$

The quality of Pretzels' protein was determined using the Protein Efficiency Ratio (PER), which was calculated according to the equation described by Alsmeyer *et al.* [27] as follows:

$$Protein\ effeciency\ ratio\ (PER) = 0.06320\ [X10] - 0.1539 \quad (4)$$

Where; X10 = threonine + valine + methionine +isoleucine + leucine + phenylalanine + lysine +histidine + arginine + tyrosine.

The biological value (B.V) was calculated according to Eggum *et al.* [28] using the following equation (5):

$$B.V\% = 39.55 + 8.89 \times lysine\ (g/100g\ protein) \quad (5)$$

2.2.11. Statistical Analysis:

Data are presented as mean values ± SD. Statistical analysis was performed using SAS [29]. One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test with $P \leq 0.05$ being considered statistically significant.

3. Results and Discussion

3.1. Chemical composition of GCS/sprouted seeds (SGC)

The chemical composition of GC seeds is presented in Table (2). GC seeds powder contained 6.69% moisture, 4.56% ash, 27.66% fat, 8.61% fiber, 21.71% protein and 30.77% carbohydrate. These results show that, the macronutrients are considerably high and suitable for human nutrition. In addition, the above findings are almost in agreement with the results reported by Vaishnavi & Gupta; Jain *et al.*[14, 30] they reported that, the garden cress seeds contain 3.25 – 5.46% moisture, 4.65 – 5.3% ash, 23.4 – 27.48% crude fat, 7.01 – 8.33% crude fiber, 21.38 – 22.81% crude protein, and 34.13 – 40.25% carbohydrate, respectively.

Based on the results illustrated in Table (2), It can be concluded that, the process of sprouting in general, whether in the soil or in the laboratory, leads to a significant ($P \leq 0.05$) increase in the levels of ash, fiber, and protein. The values of referred component were 9.89, 5.88, 10.20, 9.22, 24.71 and 25.92% for laboratory sprouted GCS after 3 and 6 days, respectively. While, the values of these components recorded 31.54, 26.30, 13.22, 11.59, 23.60 and 25.76% for soil sprouted GCS after 3 and 6 days, respectively. Garden cress seeds with higher ash concentrations are likely to be good sources of minerals. Both soil and lab. sprouting increased ash content in sprouts but the greatest increment was observed in soil garden cress sprouts, this could be due to the presence of more nutrients in the soil.

Garden cress seeds are found to contain high amount of protein. Germination is a biochemical process that involves transition of a seed from dormant state to bioactive state. It is a simple technique that has been reported to improve the nutritive value of foods. Several studies on the effect of germination on legumes have found that germination can increase protein content and dietary fiber [31]. There was significant ($p<0.05$) increase in the protein content of SGC (Lab3), SGC (Lab6), SGC (S3) and SGC (S6) by 13.81, 19.39, 8.70 and 18.65%, respectively. Also, there was a significant difference in the fiber content of sprouted garden cress seed powder. In case of germination, the fiber content has significantly ($p<0.05$) increased by 18.46, 7.08, 53.54, 34.61% for SGC (Lab3), SGC (Lab6), SGC (S3) and SGC (S6), respectively. Rajshri & Haripriya [32] reported a 38% increase in total fiber content upon germination of garden cress seeds.

On the other hand, a significant ($P \leq 0.05$) decrease in total fat values was observed for all sprouted samples. The fat content of the all sprouted garden cress samples decreased by 53.7%, 63.92%, 62.40%, and 67.32% for SGC (Lab3), SGC (Lab6), SGC (S3) and SGC (S6), respectively, compared to the raw GC powder. The decrease in fat content could be attributed to increased lipolytic enzyme activity during germination, which hydrolyses fat components into fatty acids and glycerol. [33].

The total carbohydrate content of garden cress seed GCS powder was 30.77%, significant ($P \leq 0.05$) increase was observed in lab. sprouted samples. On the other hand, significant ($P \leq 0.05$) decrease of carbohydrate content was observed in soil sprouted samples. The highest decrease ($P \leq 0.05$) was recorded in samples sprouted in soil after 3 days (15.69%). During early germination, mobilization of storage carbohydrates occurs, once the high molecular weight carbohydrates are mobilized, they are converted into soluble forms, (i.e.) Sucrose, glucose, and fructose that are readily transportable to sites where they are required for growth [34].

From the results which found in Table (2) the best sprouting of garden cress seeds was laboratory after 6 days which improved the protein content (25.92%) compared with the other sprouting conditions. So, we will complete the study their content of phenolic substances as well as their antioxidant activity for their application in the manufacture of pretzels, and its nutritional value of pretzels sample.

Table (1). The ingredients of pretzels formula

Ingredients (g)	Control	GCS			SGC		
		5%	10%	15%	5%	10%	15%
Wheat flour	100	95	90	85	95	90	85
Garden cress seed powder	-----	5	10	15	-----	-----	-----
Sprouted garden cress seed powder	-----	-----	-----	-----	5	10	15
Vegetable Oil	20	20	20	20	20	20	20
Sugar	1	1	1	1	1	1	1
Yeast	1	1	1	1	1	1	1
Table salt	1	1	1	1	1	1	1
Milk powder	4	4	4	4	4	4	4
Water	49	49	49	49	49	49	49

GCS; Garden cress seed powder SGC; Sprouted garden cress powder

Table (2). Proximate chemical composition (on dry weight) of garden cress seeds during lab. /soil sprouting.

Parameters (%)	Treatments				
	GCS	SGC (Lab3)	SGC (Lab6)	SGC (S3)	SGC (S6)
Moisture	6.69^{b}± 0.08	6.48^{b}± 0.03	8.03^{a}± 0.06	5.55^{c}± 0.08	5.70^{c}± 0.01
Ash	4.56^{e}± 0.06	9.89^{c}± 0.07	5.88^{d}± 0.05	31.54^{a}± 0.08	26.30^{b}± 0.04
Fats	27.66^{a}± 0.32	12.76^{b}± 0.28	9.98^{d}± 0.13	10.40^{c}± 0.10	9.04^{d}± 0.11
Crude Fiber	8.61^{d}± 0.60	10.20^{c}± 0.43	9.22^{d}± 0.45	13.22^{a}± 0.24	11.59^{b}± 0.53
Protein	21.71^{d}± 0.22	24.71^{b}± 0.13	25.92^{a}± 0.07	23.60^{c}± 0.12	25.76^{a}± 0.29
Total Carbohydrates	30.77^{c}± 0.84	35.96^{b}± 0.35	40.97^{a}± 0.62	15.69^{e}± 0.11	21.61^{d}± 0.79

Results are mean of three determinations ±SD. Mean values followed by different letters in the row are significantly different at $P \leq 0.05$.
GCS; garden cress seeds
SGC (Lab3); garden cress seeds sprouted in laboratory after 3 days.
SGC (Lab6); garden cress seeds sprouted in laboratory after 6 days.
SGC (S3); garden cress seeds sprouted in soil after 3 days.
SGC (S6); garden cress seeds sprouted in soil after 3 days.

3.2. Phytochemical content of GCS and SGC

Total phenolic content (expressed as mg GAE/g DW), and the total flavonoid content (expressed as mg catechin equivalent/g) of GCS and SGC methanolic extracts are shown in Table (3). The results presented in Table (3) revealed an increment in the TPC and TFC in SGC than dry seeds by 75.44 & 61.51%, respectively.

One of the best methods for enhancing the quality of seeds is sprouting. During germination, vitamins and several other substances that could be considered advantageous as antioxidants frequently undergo significant changes. Antioxidants might be defined as compounds which can prevent, delay or retard the development of rancidity or other flavour deterioration in foods or as protective factors against oxidative damage in the human body [35].In order to defend themselves against biotic pressures including illnesses, insects, and environmental challenges, plants naturally synthesize total phenolics during their growth and development. The dramatic change in phytochemicals in the germination process has been considered as natural phenomenon of plants [36]. Because the metabolism initiates in the presence of water, the particles inside the grains change, and the grains accordingly generate a great amount of energy and new compounds, including phenolics. Some studies have shown that levels of phenolics depend on extracting solvents; methanol was ideal for phenolics and flavonoids [37]. Vaishnavi and Gupta [14] have reported that Germinated garden cress seeds had the highest significant percent rise of 24.42% in comparison to raw seeds. The same trend was reported by Abdel-Aty *et al.* [6,14].

Antioxidants are categorized into two major groups, namely natural and synthetic. These groups are in dietary antioxidant intake and play major roles in maintaining the homeostasis of the oxidative balance. They are believed to protect humans from disease and aging. Synthetic antioxidants are phenolic compounds that perform the function of capturing free radicals and stopping chain reactions. They are compounds produced artificially and added to processed or pre-packaged food to prevent rancidity and browning. The most commonly used synthetic antioxidants include butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), and tertiary-butyl hydroquinone (TBHQ) [38]. However, the use of this synthetic molecule has been associated with a possible toxicity, and it has been reported that it has some side effects such as carcinogenesis, which has led to some restraint in its use [39].

The total antioxidant activity of phenolic compounds of raw garden cress seeds and their sprouts was determined using the DPPH scavenging activity method and compared with different concentrations of synthetic antioxidant (BHT) and the results are illustrated in figure (1). The antioxidant activity using DPPH significantly increased (*$P \leq 0.05$)* from seed (69.98%) to reach 90.23% in sprout, which is almost similar to the activity of a synthetic antioxidant (BHT) at the highest concentration (200ppm), where it recorded 92.22%. These results were in harmony with those reported by Abdel-Aty *et al.* [6].

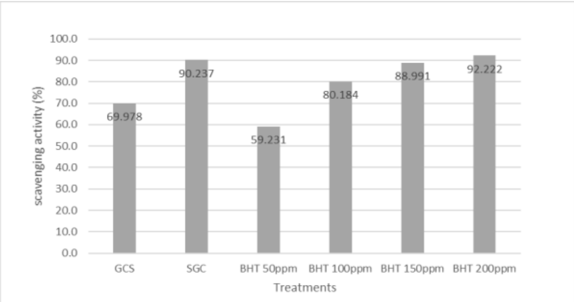

Figure 1. DPPH scavenging activity (%) of garden cress seeds (GCS) and sprouted garden cress seeds (SGC) extracts compared to different concentrations of synthetic BHT.

3.3. Sensory evaluation of prepared pretzels

Fortifying or enriching foods with a complex nutritional ingredient that are utilized in preparing dishes where there is loss during preparation might not be a wise strategy. It is recommended to complement food items like a snack, which ensures the use of all nutrients. A pretzel is one such snack with a simple food matrix with no fiber, polyphenols or other anti-nutritional factors which will reduce the benefits of added nutrients.

Garden cress seed is one of the richest sources of nutrients for health enhancing especially as source of non-heme iron [7]. Its high nutritive value and cheaper availability makes it possible for people of all sections of the society to incorporate them in their diets and enhance the nutritive quality of their meals [40]. Development of recipes for adolescents is an emerging area of interest, especially the modification and enrichment of snacks. Incorporation of cress seed in foods have shown marked increased within the iron and protein content [41].

Sensory evaluation is a unique field of evaluating consumer products using the concepts of design of experiments and statistical analysis of human senses. The comparison of the sensory attributes was made among the control and processed (raw & sprouted) garden cress samples with different levels of incorporations (5%, 10% and 15%), for pretzel (Figure 2). One-way ANOVA analysis for the sensory attributes of pretzels prepared with raw and sprouted GC powder with 5%, 10% and 15% incorporation revealed that there was a significant difference ($p<0.05$) among the experimental samples as shown in Table (4).

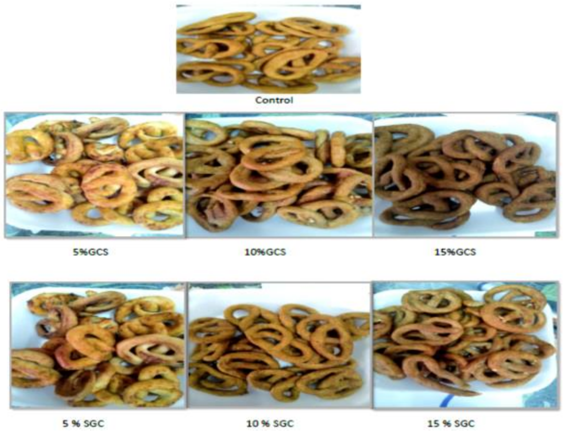

Figure 2. Pretzels sample with different substitution levels of garden cress seeds (GCS) and sprouted garden cress (SGC) compared to control.

Among the pretzels prepared from raw and sprouted garden cress, the overall acceptability scored highest for control sample then GCS (5%) substituted pretzel with a mean score of 7.9± 4.473 and 7.4± 5.087, followed by SGC (10%) substituted pretzel with a score of 7.23± 6.437. Results in Table (4) revealed a non-significant difference between GCS powder (5 & 10%) substituted pretzels, SGC powder (5&10%) substituted pretzels and control samples for most sensory attributes. On the other hand, increase the substitution level to 15%, whether in the case of GCS or SGC powder significantly ($p<0.05$) reduced scores for all sensory attributes.

From the above results, it could be concluded that wheat Pretzel samples made by replacing wheat flour with 10 % GCS powder or SGC powder show a good acceptability in sensory characteristics. Yadav *et al.* [42] incorporated untreated, dry roasted and germinated Garden cress seed powders at 2.5%, 5.5%, 8.5% and 10.5% levels to cookies. According to the sensory evaluations, the 5% integration of Garden Cress seed powder demonstrated excellent sensory qualities.

Table (3). Phytochemical contents of native and sprouted garden cress seed methanolic extracts.

Samples	Total phenolic (mg GAE/g)	Total Flavonoids (mg CE /g)
GCS	$120.330^{b} \pm 0.07$	$6.500^{b} \pm 0.71$
SGC	$211.111^{a} \pm 25.76$	$16.889^{a} \pm 2.06$

Results are mean of three determinations ±SD. Mean values followed by different letters in the column are significantly different at $P \leq 0.05$. GCS; Garden cress seeds, SGC; Sprouted garden cress.

Table (4). Effect of GCS and SGC powder substitutions on sensory parameter of wheat pretzels

Parameters	Treatments						
		GCS substitutions			SGC substitutions		
	Control	5%	10%	15%	5%	10%	15%
Appearance	$8.80^{a} \pm 0.92$	$8.20^{ab} \pm 0.59$	$7.10^{cd} \pm 0.88$	$6.40^{d} \pm 0.84$	$7.80^{bc} \pm 1.21$	$8.10^{ab} \pm 1.03$	$7.30^{bcd} \pm 1.32$
Colour	$8.70^{a} \pm 0.95$	$8.40^{a} \pm 0.66$	$7.20^{bc} \pm 0.89$	$6.40^{c} \pm 0.84$	$8.10^{a} \pm 0.70$	$8.00^{ab} \pm 1.25$	$7.80^{ab} \pm 0.89$
Odour	$8.03^{a} \pm 2.11$	$7.56^{a} \pm 2.00$	$7.30^{a} \pm 1.64$	$6.08^{b} \pm 2.54$	$7.40^{a} \pm 4.03$	$7.70^{a} \pm 2.72$	$7.40^{a} \pm 3.44$
Taste	$7.50^{a} \pm 4.08$	$7.02^{ab} \pm 4.50$	$7.02^{ab} \pm 2.51$	$5.94^{b} \pm 3.10$	$6.50^{ab} \pm 3.90$	$6.70^{ab} \pm 4.84$	$5.80^{b} \pm 5.90$
Mouth feel	$8.40^{a} \pm 0.82$	$7.80^{ab} \pm 0.56$	$7.50^{b} \pm 1.35$	$6.50^{c} \pm 1.73$	$7.40^{b} \pm 0.82$	$7.20^{bc} \pm 1.73$	$6.60^{c} \pm 1.63$
Crispness	$8.50^{a} \pm 0.74$	$7.60^{b} \pm 0.82$	$7.60^{b} \pm 1.34$	$7.50^{b} \pm 1.58$	$6.90^{c} \pm 1.18$	$7.40^{b} \pm 1.27$	$7.02^{c} \pm 1.42$
OAA	$7.90^{a} \pm 4.47$	$7.40^{a} \pm 5.09$	$7.10^{ab} \pm 3.91$	$6.30^{b} \pm 6.90$	$7.05^{ab} \pm 5.28$	$7.23^{ab} \pm 6.44$	$6.70^{b} \pm 4.22$

Results are mean of three determinations ±SD. Mean values followed by different letters in the row are significantly different at $P \leq 0.05$. GCS; Garden cress seeds, SGC; Sprouted garden cress, OAA: Overall acceptability

3.4. Proximate composition of pretzels

The perusal of Table 5 showed the proximate composition of control and supplemented pretzels. The protein content of pretzels was measured at 13.08% in the control sample and increased by 2.30, 4.28, and 13.76% with a significant difference (p 0.05) in supplemented pretzels with GCS at 5, 10, and 15%, respectively, while the increment percent reached 9.17, 11.00, and 14.45% in supplemented pretzels with SGC at 5, 10, and 15%, respectively. The same trend was observed in the other components (ash, fiber, and fats), where substitution with either GCS or SGC powders led to a significant increase ($p \leq 0.05$) in the aforementioned components with an increase in the used concentration compared to the control sample.

The available carbohydrate content of control and supplemented pretzels was calculated, and the results shown in Table (5) revealed that GCS and SGC substituted pretzels had less total carbohydrate content than the control. Previous data were consistent with those reported by Shekhara Naik *et al.*[15],where processed garden cress powder (soaking, germination, and roasting) was incorporated into a Burfi Indian product and it was found that the germination process could reduce bitterness, polyphenol content, and other anti-nutritional factors that could enhance the acceptability of SGCF-prepared products.

3.5. Mineral content of pretzel samples

Mineral content of pretzel samples is illustrated in Table (6) for control pretzels and samples with highly overall acceptability by replacing wheat flour with 10% GCS and 10% SGC. The pretzel sample which replace with 10% SGC the highest values of Zinc, iron, magnesium, calcium, potassium and phosphorus content (20.1, 67.05, 494, 1503, 2885 and 328 mg/100g respectively).

In this respect, Elizabeth and Poojara [40] in a study to modifying snacks with GCS they found that, Garden cress seeds are significantly source of iron, also minerals like calcium, zinc and phosphorus. Nathiya and Nora [41] found that, the combination of GCS in foods have shown remarkable increase in iron and protein content. Garden cress seed rich source of protein, iron, calcium, zinc and minerals content [43]. The same attitude was observed in a study by Dewidar and El-Kherbawy [44].

Germination has been shown to enhance the bioavailability of minerals such as iron and zinc in cereals and pulses Luo *et al.* [45]. Longer germination durations enhanced the hydrochloric acid extractability of calcium, iron, and zinc by 2-16%, 15-45%, and 12-25%, respectively, in pearl millet Badau *et al.* [46].

3.6. In vitro protein digestibility (IVPD) of pretzel samples

Protein digestibility was determined to provide the most effective indication of seed utilization. The results of IVPD on pretzel samples are shown in Table 7. Compared with control, 10% GCS and 10% SGC pretzel samples The IVPD of the 10% SGC pretzels sample showed the most increase, at 78.46%. This result indicated that germination could improve IVPD to a certain extent, consistent with the results of

Sharma and Gujral [47]. The increase in IVPD after germination may be a result of increased intrinsic protease hydrolysis activity, the removal of protease inhibitors, and the improvement of protein solubility [48]. Previous studies have shown that germination could activate the hydrolytic activity of the intrinsic protease in germinated grains, leading to an increase in *invitro* protein digestibility [49].

The exposed hydrophobic groups would cause proteolytic enzymes to create additional binding sites, which may hydrolyze the protein backbone and eventually increase the IVPD [50]. IVPD is an important characteristic to determine the application of proteins, which also affects the amount of protein absorbed in the body. High IVPD proteins are regarded as high-quality proteins because proteolysis makes it easier for amino acids to be released from the protein backbone, which makes them easier for the body to digest and absorb [51]. Therefore, these results demonstrated that germination could increase the utilization rate of SGC protein, improving its applications in food production. The increase in IVPD with respect to germination duration has been frequently reported previously [52-54]. Complex enzymatic processes involved in seed germination break down large macromolecules like proteins into more easily digested peptides. Partial solubilization and some proteolysis, which usually occurs during germination, improve the IVPD [55].

3.7. Amino acids profile of pretzel samples

Data presented in Table (8) show the amino acids composition of control pretzels and samples with highly overall acceptability. Essential amino acids are the amino acids that cannot be synthesized by the human body and thus, they must be obtained from the diet [26].

All essential amino acids were present in high amounts in garden cress, except tryptophan and S-containing amino acids, methionine, and cysteine. The essential amino acids score is 28.53%, with methionine being the most limiting amino acid. Glutamic acid and aspartic acid are the two major non-essential amino acids in the GCS. The total essential amino acid percentage of 47.08% indicates that this seed may considerably contribute to the supply of necessary amino acids in the diet [56].

Results of the amino acid profile for pretzels supplemented with either GCS or SGC at 10% reflect the aforementioned facts about the amino acid composition of GCS. Table 8 data show that, with the exception of methionine, all essential amino acids have increased. The highest increment in EAA (42.8, 23.07, and 20%) was found in Lysine, Arginine, and Threonine in pretzels substituted with 10% GCS and (33.3, 32.2, and 18.4%) in Lysine, Cystine, and Arginine in pretzels substituted with 10% SGC, compared to control pretzels. In contrast, methionine decreased by 13.33 and 6.66% in the 10% GCS and 10% SGC pretzel samples, respectively. Garden cress seeds have a good amount of lysine and a fair amount of tryptophan but are limited in methionine and cystine content. Thus, the value of methionine was decreased in the substituted pretzels. Chemical scores reflect the amount requirements of the essential amino acids as reported by FAO/WHO 2007. The obtained results revealed that pretzels sample substituted with SGC (10%) showed higher chemical score of valine, isoleucine, leucine and phenylalanine which were 154.29, 164.29, 128.79 and 93.65% respectively,

On the other hand, the highest increment in Non-EAA (21.05, 14.8 & 14.3%) was found in Glycine, Aspartic acid and Alanine for pretzel substituted with 10% GCS; and (66.6, 29.6 & 20%) in Tyrosine, Aspartic acid and Alanine for pretzel substituted with 10% SGC, compared to control pretzel.

Overall, substitution of pretzels with SGC resulted in a greater increase in both total essential amino acids and total non-essential amino acids compared to control sample by 14.32 and 5.59%, respectively. The previous results agree with Jain & Grover [11], who reported that the amino acid content was found to be increased in garden cress chikki as compared to the control.

The data presented in Table 8 reflected the protein quality of GCS and SGC pretzels compared to control wheat pretzels. The parameters used in the evaluation of protein quality were the protein efficiency ratio (PER) and biological value (BV). Results showed that replacing wheat flour with 10% GCS or SGC powder resulted in an improvement in the PER ratio and BV of the pretzels El-Gohery. [22].

Table (5). Chemical composition (on dry weight) of control and different substitution levels (%) of garden cress pretzels

Parameters (%)	Treatments						
	Control	GCS substitutions			SGC substitutions		
		5%	10%	15%	5%	10%	15%
Moisture	2.12^{d}± 0.01	2.28^{c}± 0.16	3.16^{b}± 0.01	4.07^{a}± 0.08	2.12^{d}± 0.01	2.18^{cd}± 0.05	1.81^{e}± 0.02
Protein	13.08^{f}± 0.02	13.38^{e}± 0.06	13.64^{d}± 0.16	14.88^{a}± 0.11	14.28^{c}± 0.01	14.52^{b}± 0.09	14.97^{a}± 0.01
Ash	1.68^{d}± 0.02	1.89^{c}± 0.09	2.15^{b}± 0.07	2.43^{a}± 0.07	1.74^{d}± 0.03	2.45^{a}± 0.05	2.45^{a}± 0.05
Crude Fiber	0.87^{e}±0.05	1.65^{d}± 0.01	2.82^{b}± 0.17	3.20^{a}± 0.21	1.80^{cd}± 0.02	1.87^{c}± 0.04	2.72^{b}± 0.01
Fats	6.94^{f}± 0.12	10.03^{c}± 0.07	10.32^{b}± 0.03	10.30^{b}± 0.01	9.66^{d}± 0.14	9.25^{e}± 0.11	10.77^{a}± 0.14
Total carbohydrates	75.33^{a}± 0.11	70.78^{b}± 0.06	67.92^{d}± 0.38	65.14^{f}± 0.32	70.43^{b}± 0.18	69.74^{c}± 0.16	67.29^{e}± 0.16

Results are mean of three determinations ±SD. Mean values followed by different letters in the row are significantly different at $P \leq 0.05$. GCS; Garden cress seeds, SGC; Sprouted garden cress.

Table (6). Mineral contents (mg/100g) of pretzels containing GCS and SGC powder.

Samples	Zn	Fe	Mg	Ca	K	P
Control	12.0^{c}±0.28	27.1^{c}±0.42	280^{c}±4.24	766^{c}±1.41	1551^{c}±1.41	232^{c}±2.83
10% GCS	16.1^{b}±0.42	37.0^{b}±0.14	430^{b}±4.24	1090^{b}±7.07	2539.5^{b}±0.71	305^{b}±2.83
10% SGC	20.1^{a}±0.28	67.05^{a}±0.21	494^{a}±0.00	1503^{a}±5.66	2885^{a}±7.07	328^{a}±4.24

Results are mean of three determinations ±SD. Mean values followed by different letters in the column are significantly different at $P \leq 0.05$.

GCS; Pretzels substituted with 10% Garden cress seeds, SGC; Pretzels substituted with 10% Sprouted garden cress

Table (7): Protein digestibility (%) of pretzel samples substituted with GCS and SGC powder.

Samples	Protein digestibility (%)
Control	62.18^{c}±0.46
10% GCS	73.86^{b}±0.07
10% SGC	78.46^{a}±0.49

Results are mean of three determinations ±SD. Mean values followed by different letters in the column are significantly different at $P \leq 0.05$.

GCS; Pretzels substituted with 10% Garden cress seeds, SGC; Pretzels substituted with 10% Sprouted garden cress

Table (8). Amino acids composition (g/100g protein) of control and garden cress pretzels

Amino acids	Control	Chemical score (%)	GCS (10%)	Chemical score (%)	SGC (10%)	Chemical score (%)	Ref. FAO/WHO (2007)
Threonine	3.50	102.94	4.20	123.53	3.90	114.71	3.4
Valine	4.60	131.43	5.10	145.71	5.40	154.29	3.5
Isoleucine	4.00	142.86	4.50	160.71	4.60	164.29	2.8
Leucine	7.60	115.15	8.30	125.76	8.50	128.79	6.6
Phenylalanine	5.20	82.54	5.60	88.89	5.90	93.65	6.3
Histidine	2.10		2.40		2.40		
Lysine	2.10	36.21	3.00	51.72	2.80	48.28	5.8
Arginine	3.80		4.70		4.50		
Cystine	3.10		3.40		4.10		
Methionine	4.50	180.00	3.90	156	4.20	168	2.5
Total EAA (%)	40.50		45.10		46.30		
Aspartic acid	5.40		6.20		7.00		
Serine	4.60		5.00		4.50		
Glutamic	35.70		35.10		35.30		
Glycine	3.80		4.60		4.50		
Alanine	3.50		4.00		4.20		
Tyrosine	2.40		3.90		4.00		
Proline	12.60		12.70		12.30		
Total NEAA (%)	68.00		71.50		71.80		
PER	2.36		2.73		2.77		
B.V.	58.22		66.22		64.44		

GCS; pretzels substituted with 10% garden cress seeds, SGC; pretzels substituted with 10% sprouted garden cress, PER; protein efficiency ratio, B.V; biological value of protein, EAA; Essential amino acids, NEAA; Non-essential amino acid.

4. Conclusions

Consumers' demand for food products that are tasty, safe, convenient, and nutritious has increased. Thus, nutrition emerged as an additional dimension in the food product development chain. Several unconventional foodstuffs have been explored, analysed, processed, and used in food product development. Garden cress (*Lepidium sativum* L.) is one such food that is brimming with not only nutrients but also health-promoting phytochemicals. On the other hand, its unpalatable flavor may be a hindrance in its nutritional applications.

Thus, based on our study, it can be concluded that sprouting reduced the bitterness of the GCS and increased the acceptability of the product developed with them. Garden cress sprouting is a very simple, inexpensive, and safe process that enhances the total phenolic and flavonoid contents and antioxidant activity several folds. Based on the findings, it can be inferred that sprouted garden cress seeds substituted for pretzels were found to be richer in proximate, amino acids, and minerals than control pretzels. Supplementation with garden cress seeds also improved the *in vitro* protein digestibility of pretzels and had well-acceptable sensory attributes.

5. Conflicts of interest

There is no conflict of interest or financial support, no funding, or financial interests/ support received during the processing of the manuscript (planning, execution, analysis).

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