Vol 2 No 4 December 2021 Pages 1-7 e-ISSN 2708-5694

Journal of Agriculture, Food and Environment (JAFE)

Journal Homepage: http://journal.safebd.org/index.php/jafe

http://doi.org/10.47440/JAFE.2021.2401

Original Article

Bioactive Compounds, Antioxidant and Antimicrobial Activity for Prepared Free Gluten Pan Bread from Sorghum Flour and Garden Cress Seeds Flour

Rania E. El-Gammal[1*], M A. Abou-Raya[1], H. A. E. F. Hassanin[2], and S. A. A. El-Bendary[2*]

[1]Food Industries Department, Faculty of Agriculture - Mansoura University, Egypt
[2]Special Foods and Nutrition Department, Food Technology Research Institution, Agriculture Research Center, Giza, Egypt

Article History

Received: 29 September 2021

Revised: 20 December 2021

Accepted: 25 December 2021

Published online: 31 December 2021

***Corresponding Author**

R. E. El-Gammal and S. A. A. El-Bendary,
E-mail: rawana@mans.edu.eg and shimaelbendary123@gmail.com

Keywords

Garden Cress Seeds Flour, Arabic Gum, Sorghum Flour, Free Gluten Pan Bread, Antioxidant Activity, Total Phenols Content, Total Flavonoids Content, shelf life, Antimicrobial Activity.

A B S T R A C T

This work was undertaken to conduct the attempt of replacing garden cress seeds flour (GCSF) with sorghum flour to produce free gluten pan bread. Shelf life and bioactive compounds (total phenolics content (TPC) and total flavonoids content (TFC) were determined. The free gluten pan bread samples were formed by partly substituting the sorghum flour by 5, 10 and 15% of GCSF. Results of bioactive compounds showed that TPC, TFC and DPPH % assay in all free gluten pan bread samples were increased in compared with the control one. All free gluten pan bread samples didn't show an observed change up to 4 days of storage under different storage condition (room and refrigerator temperature). Spoilage was pointed out by black, white and green coloration on the free gluten pan bread samples. It is concluded that substituting GCSF with sorghum flour produces acceptable free gluten pan bread with improved shelf life.

Introduction

Several studies have shown that medicinal plants are origin of various nutrient and non-nutrient molecules, many of which show antioxidant and antimicrobial properties which can preserve the body against both pathogens and cellular oxidation reactions (Wojdylo *et al.*, 2007). Garden cress seeds (*Lepidium sativum* L. (Brassicaceae (cruciferae) family)) known as 'Hab el Rashaad' or ''Thufa" locally in Egypt, are famous for its medicinal and nutritional value. Its extract contains a lot of phytochemical substances responsible for its antioxidant and antimicrobial properties as α-tocopherol, β-sitosterol, tannins, benzyl isothiocyanate, flavonoids, alkaloids, triterpenes and sterols (Abdel-Bary *et al.*, 2017). The GCS gum consists mainly of carbohydrate (77%), is a network of hydrated cellulose micelles. GCS gum is used for gluten replacement, thickening, binding, disintegrating, emulsifying, suspending, stabilizing, foaming and as gelling agents (Malviya, 2011).

Sorghum (*Sorghum bicolor* L. Moench, Poaceae family) is a tropical cultivated cereal grain. Sorghum is used into many foods, such as tortillas, baked products, gluten-free breads, composite breads, non-alcoholic or alcoholic beverages. Furthermore, sorghum is processed in various industrial applications (flour, starch, grits, malted products and flakes) (Abdelghafor *et al.*, 2011; Marston *et al.*, 2016). However, Kulamarva *et al.*, (2009) explained that because sorghum is lake in gluten, its dough and bread has needy rheological properties. To have free gluten bread with approved properties, free gluten flours were treated with different mechanism as heat treatment (Marston *et al.*, 2016), gum or hydrocolloids addition (Smith *et al.*, 2012), proteins addition (Schober *et al.*, 2011; Ziobro *et al.*, 2016), extrusion cooking (Martínez *et al.*, 2014) and heat-moisture treatment (Miyazaki and Morita, 2005). Further, Storck *et al.*, (2013) stated that egg proteins (egg albumin) addition had improved gluten-free bread properties by forming strong cohesive viscoelastic films resulting in increasing foam stability and gas retention properties. The main bioactive compounds in sorghum is phenolics (nearly all groups of phenolics), when the major phenolics compounds in sorghum are flavonoids, phenolic acids and tannins (Dykes and Rooney, 2006).

Recently, there are an increasing awareness about gluten-containing food products consumption which causes celiac disease and gluten-related disorders as non-celiac gluten sensitivity and wheat allergy (Almallouhi *et al.*, 2017). To have gluten free flours must eliminate wheat gluten or use a substitute as maize, rice, legumes, sorghum and other pseudo cereals (Giuberti and Gallo, 2018). Currently, there are various free gluten breads with low specific volume, high crumb hardness and crumbling (Hager *et al.*, 2012). So this study was attempted to produce free gluten pan bread with improved properties by using sorghum flour and garden cress seed flour (GCSF).

Materials and Methods

Materials

Raw Materials

Garden cress seeds (GCS) and Arabic gum were purchased from a certified herbal store in Cairo, Cairo governorate, Egypt. While, free gluten pan bread ingredients namely sorghum flour, sugar, dried yeast, oil, corn starch and eggs were purchased from the local market, El-Mansoura, Al-Dakhalia governorate, Egypt.

Chemicals

Dextrose, agar, sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium bicarbonate ($NaHCO_3$) and aluminum chloride ($AlCl_3$) were obtained from El-Gomhoria Company, Cairo, Cairo governorate, Egypt. While, HPLC grade methanol was purchased from Al-Shark Al-Awsat Company, Cairo, Cairo governorate, Egypt. Gallic acid, DPPH (2, 2-diphenyl-1-picrylhydrazyl), rutin and Folin-Ciocalteu reagent were purchased from Sigma–Aldrich Chemical Co. (St. Louis, USA), Cairo, Cairo governorate, Egypt.

Methods

Chemical Composition for Garden Cress Seed Flour, Sorghum Flour and Arabic Gum

Samples of Garden cress seed flour (GCSF), sorghum flour and Arabic gum were chemically analyzed to itemize the following: crude protein, moisture, crude fat, ash and crude fiber contents according to A.O.A.C. (2000), whereas total carbohydrates content was calculated by the difference.

Free Gluten Pan Bread Preparation

Sorghum grains were refined then milled, grinded and finely sieved using sieve 80 # mesh. The extraction percentage was 95%. Free gluten pan bread was prepared as mentioned by Barbone (2012) and Zannini *et al.*, (2012). Free gluten pan bread recipe was altered by partially replacing the sorghum flour by 5, 10 and 15% of garden cress seed flour (GCSF) as follows in Table (1):

Table 1. Ingredients of Free Gluten Pan Bread Prepared Replacing Garden Cress Seed Flour.

Ingredients / gm	Free Gluten Pan Bread Samples			
	Control	GCSF[1]		
		5%	10%	15%
Sorghum Flour	80	73.75	67.50	61.25
Garden Cress Seed Flour	-----	6.25	12.50	18.75
Corn Starch	20	20	20	20
Fresh Egg	30	30	30	30
Arabic Gum	4	4	4	4
Oil	10	10	10	10
Salt	2	2	2	2
Sugar	2.5	2.5	2.5	2.5
Dried Yeast	2.5	2.5	2.5	2.5
Water	70	70	80	80

[1]GCSF: Garden Cress Seed Flour.

Free gluten pan bread was prepared according to Li *et al.*, (2004) as follows:

30 g of sorghum flour were suspended in 70 ml water, which were gelatinized through heating. The resulted batter added to the rest sorghum flour amount, GCSF, corn starch, oil, fermented yeast, egg, Arabic gum, sugar and salt, and then all ingredients were mixed well forming a loose batter. Then the batter was put in a pan sized 22/ 9/ 4 cm generously greased with vegetable oil. In order to allow the dough to rise in the pan and baked at 250ºC for 40 min. the bread were removed from the pan and keep cool it at room temperature before carefully cutting. Finally, bread loaves were sliced, weighted while physical and sensory properties were estimated.

Bioactive Compounds Determination for Free Gluten Pan Bread Samples

Determination of Total Phenolics Content

The total phenolics content (TPC) of free gluten pan bread samples methanolic extracts was determined at Pesticides Laboratory, Faculty of Agriculture, Mansoura University, El-Mansoura, Al-Dakhalia governorate, Egypt, using the method described by El-Sayed *et al.*, (2017). The TPC was expressed as mg GAE/g.

Determination of Total Flavonoids Content

The total flavonoids content (TFC) of free gluten pan bread samples methanolic extracts was determined using a colorimetric assay reported by El-Sayed *et al.*, (2017) at Pesticides Laboratory, Faculty of Agriculture, Mansoura University, El-Mansoura, Al-Dakhalia governorate, Egypt. The TFC was expressed as mg RE/g.

DPPH radical scavenging activity

The antioxidant activity of free gluten pan bread samples methanolic extracts was determined using DPPH free radical scavenging method used by Akroum *et al.*, (2010) at Pesticides Laboratory, Faculty of Agriculture, Mansoura University, El-Mansoura, Al-Dakhalia governorate, Egypt. The DPPH scavenging activity was calculated from the following equation:

% DPPH scavenging activity = $[A_C - A_s \div A_C] \times 100$ where, A_C is absorbance of control and A_s is absorbance of sample.

Storage of Different Prepared Free Gluten Pan Bread Samples

The free gluten pan bread samples were stored at room temperature (25±2ºC) and refrigerator temperature (3 – 5ºC) and were observed for 12 days. The stored samples were visually observed for fungi growth according to Ijah *et al.*, (2014).

Total Fungi Count

The total fungi count of free gluten pan bread samples was counted at Microbiology Laboratory, Faculty of Agriculture, Mansoura University, El-Mansoura, Al-Dakhalia governorate, Egypt. After incubation, the number of spores per gram of a sample was found out using the method represented by Aneja (2003), Jay (2005) and Saeed *et al.*, (2018).

Determination of Antimicrobial Activity of Methanol Extracts of Sorghum, Garden Cress Seed and Arabic Gum

Antimicrobial activity of the methanol extracts of sorghum flour, garden cress seed flour (GCSF) and Arabic gum against isolated organisms was done by the agar-well diffusion method according to Kingsbury and Wagner (1990) at Microbiology Laboratory, Faculty of Agriculture, Mansoura University, El-Mansoura, Al-Dakhalia governorate, Egypt. After incubation, the diameters of the inhibition zones were measured to the nearest mm. Tow replicates were done for each concentration. The antimicrobial activity of the extracts against the isolated microorganisms was compared with that of DMSO as solvent control.

Statistical Analysis

Data were analyzed statistically by analysis of variance, for statistical significance ($P \leq 0.05$) using LSD test (one way ANOVA) according to Steel *et al.*, (1997), using the statistical program CoStat (Ver. 6.303).

Results and Discussion

Chemical Composition for Garden Cress Seed Flour, Sorghum Flour and Arabic Gum.

Chemical composition for garden cress seed flour (GCSF), sorghum flour and Arabic gum is illustrated in Table (2). According to the LSD analysis method, data showed that sorghum flour contain as mean ± SD 12.01 ± 2.16 g/100g crude protein, 4.12 ± 0.37 g/100g crude fat, 9.60 ± 1.92 g/100g moisture, 2.10 ± 0.76 g/100g ash, 2.99 ± 2.21 g/100g crude fiber and 78.78 ± 4.90 g/100g carbohydrates (by difference). While, results indicated that GCSF was scored statistically the highest amount of crude protein (19.90 ± 9.82 g/100g) among all samples. When, there was no significant difference observed between sorghum flour and GCSF 12.01 ± 2.16 and 19.90 ± 9.82 g/100g, respectively. Where, Arabic gum was scored statistically the lowest amount of crude protein (2.36 ± 0.91 g/100 g) among all samples. These data are in agreement with those demonstrated by Zia-Ul-Haq *et al.*, (2012) who showed that GCSF was scored 24.2 ± 0.5% of crude protein. Whereas, Adeyeye (2016) reported that sorghum flour was scored 10.72 ± 0.24 % of crude protein. Also, Mansoori *et al.* (2020) exhibited that Arabic gum was scored 2.50 ± 1.07 % of crude protein.

Table 2. Chemical Analysis of Garden Cress Seed Flour, Sorghum Flour and Arabic Gum.

Samples	Crude Protein (g/100g)	Crude Fat (g/100g)	Moisture (g/100g)	Ash (g/100g)	Crude Fiber (g/100g)	Carbohydrates (g/100g)
Sorghum Flour	12.01 ± 2.16^{a}	4.12 ± 0.37^{b}	9.60 ± 1.92^{b}	2.10 ± 0.76^{b}	2.99 ± 2.21^{b}	78.78 ± 4.90^{a}
GCSF[1]	19.90 ± 9.82^{a}	19.77 ± 10.23^{a}	4.64 ± 1.81^{c}	8.65 ± 2.65^{a}	9.36 ± 2.53^{a}	42.32 ± 16.36^{b}
Arabic Gum	2.36 ± 0.91^{c}	0.25 ± 0.11^{b}	12.23 ± 0.86^{a}	2.68 ± 0.69^{b}	0.73 ± 1.93^{b}	93.98 ± 0.02^{a}

[1]GCSF: Garden Cress Seed Flour.
Each value is the mean of 3 replicates ± SD.
All values on dry weight basis.
Values in the same column with different superscript letters (a, b,….) are significantly different ($P \leq 0.05$).

Concerning the crude fat, Table (2) demonstrated that GCS flour was scored statistically the highest amount of crude fat 19.77 ± 10.23 g/100g among all samples. When, there was no significant difference observed between sorghum flour and Arabic gum (4.12 ± 0.37 and 0.25 ± 0.11 g/100g, respectively), when compared to GCSF recorded statistically the lowest content. These data are matched with those represented by Zia-Ul-Haq *et al.*, (2012) who reported that GCS flour was scored 23.2 ± 0.2 % of crude fat. Whereas, Adeyeye (2016) reported that sorghum flour was scored 3.83 ± 0.21 % of crude fat. Also, Mansoori *et al.* (2020) reported that Arabic gum was scored 0.14 ± 0.01 % of crude fat.

In terms of moisture, results stated that Arabic gum was observed statistically the highest amount of moisture (12.23 ± 0.86 g/100g) among all samples. Where, GCSF was scored statistically the lowest amount of moisture (4.64 ± 1.81 g/100g). In harmony, Mansoori *et al.* (2020) reported that Arabic gum was recorded 9.17 ± 0.19 % of moisture. Parallel, Adeyeye (2016) mentioned that sorghum flour moisture content was 10.28 ± 0.39. These results matched with those of Zia-Ul-Haq *et al.*, (2012) who stated that GCSF was scored 2.9 ± 0.1% of moisture.

With regard to ash content data presented in Table (2) indicated that there was no significant difference observed between sorghum flour and Arabic gum. Where, GCSF was scored statistically the highest amount 8.65 ± 2.65 g/100g of ash content. These data are in conformity with those described by Zia-Ul-Haq *et al.*, (2012) who reported that GCSF was recorded 7.1 ± 0.1% of ash content. As, Adeyeye (2016) reported that sorghum flour was observed 2.41 ± 0.19 of ash content. Also, Mansoori *et al.* (2020) reported that Arabic gum was scored 3.11 ± 0.17 % of ash content.

Concerning crude fiber, results showed that GCSF was scored statistically the highest amount of crude fiber (9.36 ± 2.53 g/100g) among all samples. When, there was no significant difference observed between sorghum flour and Arabic gum (2.99 ± 2.21 and 0.73 ± 1.93 g/100g, respectively). These results are in accordance with those by Adeyeye (2016) who reported that sorghum flour was recorded 2.32 ± 0.14 % of crude fiber. In harmony, Zia-Ul-Haq *et al.*, (2012) reported that GCSF crude fiber content was 11.9 ± 0.4 %.

Regarding the carbohydrates content, Table (2) displayed that there was no significant difference observed between sorghum flour and Arabic gum (78.78 ± 4.90 and 93.98 ± 0.02 g/100g, respectively), when recording statistically the highest carbohydrates content. While, GCSF was scored statistically the lowest amount of carbohydrates 42.32 ± 16.36 g/100g between all samples. These results are matched with those by Zia-Ul-Haq *et al.*, (2012) who reported that GCSF was observed 30.7 ±1.2% of carbohydrates. Also, Mansoori *et al.* (2020) reported that Arabic gum was scored ~85.08 % of carbohydrates.

Effect of Garden Cress Seed Flour Replacing on Bioactive Compounds of Different Prepared Free Gluten Pan Bread Samples

Total phenolic compounds content, total flavonoids content and DPPH % radical scavenging activity of different prepared free gluten pan bread samples are illustrated in Table (3). It is observed that total phenolic content (TPC) was gradually increased significantly with the replacing of GCSF. Table (3) performed that the highest amount of TPC was observed in free gluten pan bread sample with 15% (2.24 ± 0.17 mg GAE/g). While, the lowest amount of TPC was in free gluten pan bread control sample (0.89 ± 0.08 mg GAE/g).

Table 3. Effect of Garden Cress Seed Flour Replacing on Bioactive Compounds of Different Prepared Free Gluten Pan Bread Samples.

Pan Bread Samples		Total Phenolic Content mg GAE/g	Total Flavonoid Content mg RE/g	DPPH Radical Scavenging Activity %
Control		0.89 ± 0.08^{c}	0.44 ± 0.14^{c}	44.94 ± 8.76^{b}
GCSF[1]	5%	1.46 ± 0.13^{b}	3.91 ± 0.12^{b}	92.35 ± 0.32^{a}
	10%	1.70 ± 0.12^{b}	5.65 ± 0.33^{a}	82.66 ± 0.95^{a}
	15%	2.24 ± 0.17^{a}	0.92 ± 0.10^{c}	87.73 ± 1.11^{a}

[1]GCSF: Garden Cress Seed Flour.
Each value is the mean of 2 replicates ±SD.
Values in the same column with different superscript letters (a, b,.) are significantly different (P≤ 0.05).

Respecting total flavonoids content (TFC) was increased significantly with the replacing of GCSF. Results exhibited that the highest amount of TFC was observed in free gluten pan bread sample with 10% (5.65 ± 0.33 mg RE/g). While, the lowest amount of TFC was in free gluten pan bread control sample (0.44 ± 0.14 mg RE/g). Table (3) figured out that DPPH % radical scavenging activity was increased significantly with the replacing of GCSF. Thus, the highest content of DPPH % was observed in free gluten pan bread samples with 5, 10 and 15% GCSF (92.35 ± 0.32, 82.66 ± 0.95 and 87.73 ± 1.11 %, respectively). While, the lowest content of DPPH % was in free gluten pan bread control sample (44.94 ± 8.76 %). Further, there was no significant difference observed between 5, 10 and 15% GCSF (92.35 ± 0.32, 82.66 ± 0.95 and 87.73 ± 1.11, respectively).

Shelf Life of Different Prepared Free Gluten Pan Bread Samples Prolonged Storage Period (12 Days)

Different prepared free gluten pan bread samples were stored up to 12 days at room (30ºC) and cooled (3 – 5ºC) temperature. Shelf life of different prepared free gluten pan bread samples prolonged storage period (12 days) is illustrated in Table (4). Results stated that all free gluten pan bread samples didn't show an observed change up to 4 days of storage under different storage condition (room and refrigerator temperature). Spoilage was pointed out by yellow, black and green coloration on the free gluten pan bread samples. It is noticed that free gluten pan bread lasted for 4 – 5 days of storage at room temperature and for 6 – 11 days of storage at refrigerator temperature.

Table 4. Shelf Life of Different Prepared Free Gluten Pan Bread Samples Prolonged Storage Period (12 Days).

Pan Bread Samples		Shelf Life (days)	
		Room Temperature	Refrigerator Temperature
Control		5	9
GCSF[1]	5%	4	6
	10%	5	6
	15%	4	11

[1]GCSF: Garden Cress Seed Flour.

In accordance, Ijah *et al.*, (2014) mentioned that bread lasted for 6–8 days before noticing obvious spoilage, indicated by yellow, black and green coloration. And found that mold growth was consisted of *Penicillium* sp., *Aspergillus flavus*, *Mucor mucedo* and *Rhizopus stolonifer*. Explanatory Shama *et al.*, (2011) demonstrated that GCS seeds contain benzyl isothiocyanate, flavonoids, tannins, triterpens, alkaloids, sterols and glucosinolates, which exhibited an antimicrobial effect. Particularly, Tannins inhibit protein synthesis by building an irreversible compound with proline-rich proteins. Abstractly, Rana and Kaur (2016) stated that preservatives stabled bakery products against fungi attack, helped to minimize food wastage caused by microorganism spoilage. Thus, preservatives usage resulted in longer shelf life for bakery products stored at store and home.

Effect of Storage Conditions on Total Fungi Count for Pan Bread Samples

Results of free gluten pan bread samples for total fungi count during a storage period of 12 days are shown in Table (5). Table (5) demonstrated that there was no growth observed at the first day of storage for all samples at room and cooling temperature. In harmony, Unachukwu and Nwakanma (2015) exhibited on the first two days of storage that there was no growth observed for all samples. Also, Badr (2015) observed that free gluten pan bread samples have no detected growth at zero time of storage.

Table 5. Effect of Storage Conditions on Total Fungi Count for Pan Bread Samples.

Pan Bread Samples		Room Temperature (cfu/g)			Refrigerator Temperature (cfu/g)		
		Day 1	Day 7	Day 12	Day 1	Day 7	Day 12
Control		NG	0.1×10^{2}	30.6×10^{2}	NG	NG	12.9×10^{2}
GCSF[1]	5%	NG	7.5×10^{2}	111×10^{2}	NG	0.1×10^{2}	2×10^{2}
	10%	NG	4×10^{2}	54×10^{2}	NG	2.1×10^{2}	11.1×10^{2}
	15%	NG	40×10^{2}	81×10^{2}	NG	NG	10.3×10^{2}

[1]GCSF: Garden Cress Seed Flour. *NG: No growth.
Each value is the mean of 2 replicates.

Also, in Table (5) free gluten breads total fungi count was increased significantly with increasing GCSF replacing. Results of total fungi count was increased at day 7 at room temperature for free gluten pan bread sample with 5 and 15% GCSF (7.5×10^{2} and 40×10^{2} cfu/g, respectively). Concerning total fungi count at day 7 of refrigerator storage, there was no growth count observed for free gluten pan bread control sample and free gluten pan bread sample with 15% GCSF. While, total fungi count was increased at day 7 at cooling temperature for free gluten pan bread samples with 5 and 10% GCSF (0.1×10^{2} and 2.1×10^{2} cfu/g, respectively).

Day 12 at room and refrigerator temperature the growth was increased rapidly. Results noticed that at room temperature at free gluten pan bread sample with 5 and 15% GCSF was counted 111×10^2 and 81×10^2 cfu/g, respectively when compared to the control one (30.6×10^2 cfu/g). Where, the free gluten pan bread sample with 5% has observed the highest count 111×10^2 cfu/g. In addition, 12 days of refrigerator temperature storage free gluten pan bread sample with 5, 10 and 15% GCSF was counted 2×10^2, 11.1×10^2 and 10.3×10^2 cfu/g, respectively when compared to the control one (12.9×10^2 cfu/g). Where, the free gluten pan bread control sample has observed the highest count 12.9×10^2 cfu/g.

In accord, Unachukwu and Nwakanma (2015) demonstrated that bread over a storage period of 7 days had a fungal range of 6-8 x 10^3 cfu. With increasing storage period fungal count grew. Day 7 recorded the highest fungal count. Unachukwu and Nwakanma (2015) isolated *Mucor spp, Aspergillus spp, Fusarium spp, Penicillium spp and Rhizopus spp* from stored bread. These data are in agreed with Badr (2015) who determined total mold count during 12 days storage period at room temperatures in pan bread partially substituted of wheat flour with watermelon rind powder (WMRP) levels (3, 6, 9 and 12 %). And noted that control pan bread sample counted higher mold count (2.1, 3.2 and 5.2 log cfu /g) at 2, 4 and 6 days, respectively. While, pan bread samples with 3, 6, 9 and 12 % WMRP (1.7, 2.3, 2.8 and 5.2 log cfu /g) after 2, 4, 6 and 8 days, respectively. Therefore, Badr (2015) concluded that the reduction of mold count may be ascribed to replacement with watermelon rind powder containing high level of phenolic compound which inhibit or kill microbial growth and subsequently has a reduced microorganism growth and a slow increase in microbial numbers, leading to increasing the antioxidant potential and shelf life with accepted sensory quality.

Antimicrobial Activity of Methanol Extracts of Sorghum, Garden Cress Seed and Arabic Gum against Isolated Organisms

Antimicrobial activity of methanol extracts of sorghum, garden cress seed and Arabic gum against isolated organisms using agar well diffusion method at the different concentrations 50, 100, 200 and 400% are illustrated in Table (6). Table (6) showed that GCS extract was more effective in inhibition of F1 especially 200 concentration. The results indicated that the concentrations 200 and 400% of sorghum extract and GCS extract in all concentrations were active against B1, B3, B5 and B6. Therefore, F1, F2, B1, B3, B5, B6 and B7 are susceptible to GCS extract at the concentration 200%. The most susceptible microorganism to sorghum extract was B6 at 50, 100, 200 and 400% concentration. In addition, sorghum extract showed moderate inhibitory action to F1 and B7. It can be suggested that F1, F2 and B7 were the most resistant organisms to the concentrations 50, 100, 50, 100, 200 and 400%. DMSO has poor inhibitory effect against F1, F2, B1, B3 and B5. 200 and 400% of the sorghum extract. Maximum activity of sorghum extract was seen against B6 at concentration. In the present findings, F1, B6 and B7 were found susceptible to sorghum extract. On the other hand, Arabic gum extract found to have significant antimicrobial activity against F1, B1, B5 and B7. In harmony, Chatoui *et al.*, (2016) reported that the 1% DMSO has no zone of inhibition in vitro disc diffusion method. *Lepidium sativum* seeds methanol extract has 13.15 mm zone of inhibition against the *Rhodococcus equi* germ, due to containing antibacterial components, alkaloids, and saponosides. Where, Sudan *Lepidium sativum* seeds methanol extract has vigorous antibacterial activity against *E. coli, S. aureus, Proteus vulgaris, Klebsiella pneumonae and Pseudomonas aeruginosa* at 2.5, 5 and 10% concentrations. While, Ethiopian *Lepidium sativum* seeds methanol extract has a fine zone of inhibition against *B. subtillis, E. coli. and S. aureus,* due to containing flavonoid and tannin.

Table 6. Antimicrobial Activity of Methanol Extracts of Sorghum, Garden Cress Seed and Arabic Gum against Isolated Organisms.

Samples	Extract Concentration mg/ml	Inhibition Zone (mm)						
		Fungi		Bacteria				
		F 1	F 2	B 1	B 3	B 5	B 6	B 7
Control (DMSO)	------	0	0	0	0	0	9	5
Sorghum Extract	50	12	5	6	9	7	10	6
	100	8	9	7	9	13	12	2
	200	9	0	13	11	12	14	4
	400	5	0	12	12	14	11	6
GCS[1] Extract	50	11	13	10	12	13	11	9
	100	10	0	9	12	13	10	11
	200	18	10	15	10	12	10	12
	400	8	9	17	11	13	12	15
Arabic Gum Extract	50	0	0	0	0	0	0	0
	100	3	0	4	0	0	0	0
	200	5	0	7	0	3	0	4
	400	6	0	9	0	6	0	10

[1]GCS: Garden Cress Seed.
Each value is the mean of 2 replicates.

In parallel, Abo El-Maati *et al.*, (2016) found that *Lepidium sativum* seeds (microwave assist and ultrasonic assist) extracts were caused zones of inhibition against *Escherichia coli* (9-12), *Staphylococcus aureus* (8-10 mm), *Salmonella Enteritidis* (15-20 mm), *Serratia marcescens* (9 and 7 mm) and *Listeria monocytogenes ScottA* (10 mm). These results are in accordance with Adam *et al.*, (2011) who tested garden cress seed extract against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Candida albicans*. In agreed, Abo El-Maati *et al.*, (2016) found that garden cress seed extract has a variant antioxidant activity in a dose-dependent manner, due to higher phenolics content, which is comparable to TBHQ, possibly using garden cress seed as food preservative or in pharmaceutical industries.

Conclusion

The replacing of garden cress seeds flour to sorghum flour led to improve the antioxidant activity of free gluten pan bread and increasing the shelf life of the stored free gluten pan bread at room temperature and at refrigerator temperature due to its increased phenolic compounds content. Based on antioxidant activity results, garden cress seeds flour was increased the total phenolics content, total flavonoids content and DPPH % radical scavenging activity. These results indicated that free gluten pan bread sample containing 15% GCSF was improved its shelf life. Based on antioxidant activity and microbiological analysis, free gluten pan bread sample with 15% GCSF was attained optimum properties and fitted for functional bread development. Generally, the replacing of garden cress seeds flour had a positive effect on increasing storage stability and bread quality. Replacing of garden cress seeds flour, as gluten substitute and bread improver, had an overall positive effect on sorghum - garden cress seeds based free gluten pan bread. This research provides an improved quality and longer lasting free gluten pan bread.

References

Abdel Bary, E.M.; Fekri, S.; Yaser, A. and Harmal, A.N. (2017). Novel superabsorbent membranes made of PVA and Ziziphus spinachristi cellulose for agricultural and horticultural applications. New J. Chem. 41: 9688–9700.

Abdelghafor, R.; Mustafa, A.; Ibrahim, A. and Krishnan, P. (2011). Quality of bread from composite flour of sorghum and hard white winter wheat. Adv. J. Food Sci. Technol. 3: 9-15.

Abo El-Maati, M.F.; Labib, S.M.; Al-Gaby, A.M.A. and Ramadan, M.F. (2016). Antioxidant and Antibacterial Properties of Different Extracts of Garden Cress (*Lepidium Sativum* L.). Zagazig J. Agric. Res. 43(5): 1685-1697.

Adam, S.I.Y.; Salih, S.A.M. and Abdelgadir, W.S. (2011). In vitro antimicrobial assessment of *Lepidium sativum* L. Seeds extracts. Asian J. Med. Sci. 3(6): 261–266.

Adeyeye, S.A.O. (2016). Assessment of quality and sensory properties of sorghum–wheat flour cookies. Cogent Food Agri. 2: 1245059.

Akroum, S.; Bendjeddou, D., Satta, D. and Lalaoui, K. (2010). Antibacterial, antioxidant and acute toxicity tests on flavonoids extracted from some medicinal plants. Int. J. Green Pharm. 4(3):165-169.

Almallouhi, E.; King, K.S.; Patel, B.; Wi, C.; Juhn, Y.J.; Murray, J.A. and Absah, I. (2017). Increasing incidence and altered presentation in a population-based study of pediatric celiac disease in North America. J. Pedia. Gastro. Nutri. 65(4): 432–437.

Aneja, K. (2003). Experiments in microbiology, plant pathology and biotechnology. 4th Ed., New Delhi: New Age International Publishers: 607.

A.O.A.C. (2000). Official Methods of analysis of Association Official Chemists. 15th Ed., Washington. D. C. U.S.A.

Badr, S. (2015). Quality and Antioxidant Properties of Pan Bread Enriched with Watermelon Rind Powder Current Science International. Curr. Sci. Int. 4(1): 117-126.

Barbone, E. (2012). How to cook gluten-free. Lake Isle Press New York, NY, 204.

Chatoui, K.; Talbaoui, A.; Aneb, M.; Bakri, Y.; Harhar, H. and Tabyaoui, M. (2016). Phytochemical Screening, Antioxidant and Antibacterial activity of *Lepidium sativum* seeds from Morocco. J. Mater. Environ. Sci. 7(8): 2938-2946.

Dykes, L. and Rooney, L.W. (2006). Sorghum and millet phenols and antioxidants. J. Cereal Sci. 44: 236–251.

El-Sayed, M.M.; Hashash, M.M.; Abdel-Hady, A.A.; Abdel-Hady, H.; Abdel-Lateef, E.E. and Morsi, E.A. (2017). Total phenolic and flavonoid contents and antioxidant activity of *Lantana camara* and *Cucurbita pepo* (Squash) extracts as well as GC-MS analysis of *Lantana camara* essential oil. World J. Pharm. Res. 6(1): 137-153.

Giuberti, G. and Gallo, A. (2018). Reducing the glycemic index and increasing the slowly digestible starch content in gluten-free cereal-based foods: A review. Int. J. Food Sci. Technol. 53(1): 50–60.

Hager, A.S.; Wolter, A.; Czerny, M. and Bez, J. (2012). Investigation of product quality, sensory profile and ultrastructure of breads made from a range of commercial gluten-free flours compared to their wheat counterparts. Eur. Food Res. Technol. 235: 333–344.

Ijah, U.J.J.; Auta, H.S.; Aduloju, M.O. and Aransiola, S.A. (2014). Microbiological, Nutritional, and Sensory Quality of Bread Produced from Wheat and Potato Flour Blends. Int. J. Food Sci. Article ID 671701.

Jay, M. (2005). Modern food microbiology. 4th Ed. New York: Chapman and Hall. 187.

Kingsbury, D. and Wagner, G. (1990). Microbiology. Williams and Wilkins. Maryland. 36.

Kulamarva, A.G.; Soslea, V.R. and Vijaya Raghavana, J.S. (2009). Nutritional and Rheological Properties of Sorghum. Int. J. Food Prop. 12: 55-69.

Li, F.D.; Li, L.T.; Li, Z. and Tatsumi, E. (2004). Determination of Gelatinization Temperature of by Ohmic Heating. J. Food Engine. 62: 113-120.

Mahassni, S.H. and Al-Reemi, R.M. (2013). Apoptosis and necrosis of human breast cancer cells by an aqueous extract of garden cress (*Lepidium sativum*) seeds. Saudi J. Bio. Sci. 20: 131–139.

Mansoori, N.; Majzoobi, M.; Gavahian, M.; Badii, F. and Farahnaky, A. (2020). Acacia Gum as a Natural Anti-Plasticizer for the Production of Date Syrup Powder: Sorption Isotherms, Physicochemical Properties, and Data Modeling. Foods. 9 (50): 1 – 17.

Marston, K.; Khouryieh, H. and Aramouni, F. (2016). Effect of heat treatment of sorghum flour on the functional properties of gluten-free bread and cake. LWT-Food Sci. Technol. 65: 637-644.

Martínez, M.M.; Oliete, B.; Roman, L. and Gomez, M. (2014). Influence of the addition of extruded flours on rice bread quality. J. Food Qual. 37: 83-94.

Miyazaki, M. and Morita, N. (2005). Effect of heat-moisture treated maize starch on the properties of dough and bread. Food Res. Int. 38: 369-376.

Rana, R. and Kaur, P. (2016). Sensory and Nutritional Evaluation Of Food Products Enriched With Germinated Garden Cress Seed Flour. Int. J Food Nutri. Sci. 5(4): 96 – 101.

Saeed, I.; Shaheen, S.; Hussain, K.; Asaf Khan, M.; Jaffer, M.; Mahmood, T.; Khalid, S.; Sarwar, S.; Tahir, A. and Khan, F. (2018). Assessment of mold and yeast in some bakery products of Lahore, Pakistan based on LM and SEM. Microsc. Res. Tech. 1–7.

Schober, T.J.; Bean, S.R.; Tilley, M.; Smith, B.M. and Ioerger, B.P. (2011). Impact of different isolation procedures on the functionality of zein and kafirin. J Cereal Sci. 54: 241–249.

Shama, I.Y.; Adam, S.; Salih, A.M.; Warda, S. and Abdelgadir, A. (2011). In vitro Antimicrobial Assessment of *Lepidium sativum* L. Seeds Extracts. Asian J. Med. Sci. 3(6): 261-266.

Smith, B.; Bean, S.; Aramouni, F. and Herald, T.J. (2012). Effect of HPMC on the quality of wheat-free bread made from carob germ flour-starch mixtures. J. Food Sci. 77: 684– 688.

Steel, R.; Torrie, J. and Dickey, D. (1997). Principles and procedures of Statistics: Biometrical Approach. 3rd Ed., McGraw-Hill, New York, NY.

Storck, C.R.; da Rosa Zavareze, E.; Gularte, M.A.; Elias, M.C.; Rosell, C.M. and Dias, A.R.G. (2013). Protein enrichment and its effects on gluten-free bread characteristics. LWT-Food Sci. Technol. 53(1): 346–54.

Unachukwu, M.N. and Nwakanma, C. (2015). The fungi associated with the spoilage of bread in Enugu state. Int. J. Curr. Microbiol. App. Sci. 4(1): 989-995.

Wadhwa, S.; Panwar, M.S.; Agrawal, A.; Saini, N. and Patidar, L.N. (2012). A review on pharmacognostical study of *lepidium sativum*. Adv. Res. Pharma. Bio. 2(IV): 316–323.

Wojdylo, A.; Oszminaski, J. and Aviram, M. (2007). Food Chem. 105: 940 – 949.

Zannini, E.; Jones, J.; Renzetti, S. and Arendt, E. (2012). Functional replacements for gluten. Ann. Rev. Food Sci. Technol. 3: 227 – 245.

Zia-Ul-Haq, M.; Ahmad, S.; Calani, L.; Mazzeo, T.; Rio, D.D.; Pellegrini, N. and Feo, V.D. (2012). Compositional study and antioxidant potential of Ipomoea Hederacea Jacq and *Lepidium sativum* L. Seeds. Molecule. 17: 10306 – 10321.

Ziobro, R.; Juszczak, L.; Witczak, M. and Korus, J. (2016). Non-gluten proteins as structure forming agents in gluten free bread. J. Food Sci. Technol. 53: 571– 580.