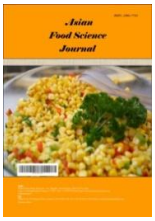

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Effect of Fortification Breadsticks with Milk Thistle Seeds Powder on Chemical and Nutritional Properties

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Authors' contributions

This work was carried out in collaboration among all authors. Author GSEH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GSEH and EAY managed the analyses of the study. Author ASAES managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This work aim to verify the importance of milk thistle seed powder (MTSP) as a medicinal plant and effect of fortification wheat flour (WF) with MTSP on chemical and nutritional properties. Therefore, the chemical composition such as ether extract, dietary fiber, crude protein, vitamin C, amino acids and minerals contents were determined. Also, the antioxidative properties such as phenolic compounds and flavonoid compounds and scavenging activity on DPPH free radicals were also determined. Furthermore, the possibility to use MTSP in bread sticks preparation, where the use of MTSP as replacer of WF at different extents (4, 8 and 12%) to prepare bread sticks rich in protein and minerals, as well as organoleptic evaluation of prepared bread sticks were investigated. Results indicated that MTSP have high amount of protein (25%), ether extract (25.56%), crude fiber (29.68%), minerals content (4.55%), vitamin C (4.59 mg/g), flavonoids (20,65 mg/g), and phenolic compounds (40.33 mg as Gallic /g) (antioxidants) which, could be used as alternative

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natural source for synthetic antioxidants in industry. The indispensable and dispensable amino acids are more abundant in MTSP protein. Sensorial evaluation of the obtained products indicated that successful replacement was 4, 8 and 12% of MTSP. So, it could be recommended to use the investigated plant seeds in bread sticks production for its nutritional concept and antioxidative effects. Finally, the use of inexpensive available natural sources of bioactive material and incorporated to our low-cost foods for treatment or protection of our widespread disease is innovated road to produce low price therapeutic foods available to wide levels of our people.

Keywords: Seeds; bread sticks; protein; phenol; flavonoids.

1. INTRODUCTION

Milk thistle plant *Silybum marianum*, family: Compositae is an annual herb, natural to the Mediterranean region, which these days has spread out to the warm and dry regions of Southern Europe and Asia. It is cultivated a medicinal plant, as an ornamental, species, and as a minor vegetable. MTSP has been used in popular medicine for over 2,000 years as a remedy for a variety of medical conditions, particularly gallbladder, liver and kidney ailments [1]. The roots of MTSP can be eaten boiled, fried or raw fried. In spring, young sprouts can be cut down to their roots and boiled. Thorny bracts were eaten in the past as a substitute to artichokes, leaves of herb can be cooked to get an appropriate additional of spinach and can also be mixed into fresh salads and stems (after cleaning) were soaked above 12 h in water to remove their bitterness and at that point fried. MTSP are the important source of silymarin 4 to 6% from weight seeds, with uses in the medicinal industry. Silymarin is a flavonoid compound comprising numerous flavonolignans, comprising isosylibinin, silybinin, silychristin and silydianin. [2]. El-haak et al. [3] stated that MTSP contain 29.68%crude ether extract, 25.25% proteins, 38.16% carbohydrates and 29.95% dietary fibre. Additionally, proteins of MTSP contained have markedly high quantities of indispensable amino acids for example threonine, valine, isoleucine, leucine, and lysine.

MTSP comprises a comparatively high quantity of ether extract, which makes one step extraction of silymarin from the seed difficult. Whereas, oil is residual of silymarin industrial manufacture and has to be removed from MTSP [4].

Although the defatted MTSP is a residual and is usually removed as waste, it may comprise valuable constituents such as carbohydrates (especially crude fibres), proteins, minerals and some bioactive compounds that have anti-microbial or antioxidant properties [5]. MTSP are better known as having therapeutic benefits. MTSP can be consumed as food innovation. It is a great stimulating, increases aids in digestion and appetite. MTSP is a main source of minerals such as Fe, Mg, Ca and P [6]. Fe is more readily absorbed in the ferrous state (Fe^{2+}) but greatest of the dietary Fe is in the ferric form (Fe^{3+}) [7]. Also, iron absorption is effected of several factors such as ascorbic acid, protein and carbohydrate consumptions improve absorption [8,9]. On the contrary, binding agents such as oxalate, phosphate, phytate, Ca, tea, dietary fiber, coffee, gastrointestinal illnesses inhibit Fe absorption [10].

The importance of MTSP in human nutrition will also increase, as increasing number of peoples are looking for foodstuffs with pro-health properties [11]. Moreover, [12,13] were studied the influence of MTSP mixing on bread quality. Furthermore, [14] showed that the extents of silymarin mixed were carefully chosen to be not upper than 10% of medicinal dose.

The purpose of the current study was to substitute WF in bread sticks by MTSP to enhance nutritious value and to enhance sensorial attributes of bread sticks consequently to get a high quality product.

2. MATERIALS AND METHODS

2.1 Materials

Milk thistle seeds (*Silybum marianum*), wheat flour (*Triticum aestivum*), instant active dry yeast, salt (sodium chloride), oil and sugar (sucrose) were purchased from the local market, Cairo city, Egypt. The chemicals and solvents were used in this study were analytical grade and Sigma Company.

2.1.1 Milk thistle seeds powder preparation

MTSP were milled electrically in laboratory mill by Brown Multiquick blender (Germany) at speed 2 for 3 min to pass through 60 mesh sieve, then MTSP was packed in polyethylene bags and stored at -18°C freezing until used [15].

2.2 Methods

2.2.1 Bread stick preparation

Processing of bread sticks enriched with MTSP as shown Table 1.

The straight dough formulas were used for bread sticks preparing as follow: oil, sugar, salt and instant dry yeast, were added to each types of flour with warm water and oil, the materials were carefully mixed together by hand. Dough was left to ferment for 1/2 hour at room temperature (30 ± 2°C). Formulas of dough was cut into small portions and kept for ten min to rest. The fermented sticks were shaped to the final form and fermented for 30 min at 30°C and 90% relative humidity. Fermented snacks doughs were baked at 170°C for 1/2 hour [16].

2.2.2 Chemical composition

Ether extract (official method no 935.38) using petroleum ether (60-80°C) in a Soxhlet apparatus, total nitrogen following kjeldahle method (official method no 950.36), fibers of samples (official method no 950.37), ash content (official method no 930.22) in muffle furnace at 450 - 500°C and carbohydrates were calculated by procedures outlined by AOAC [17].

2.2.3 Estimation of minerals

Minerals containing potassium, calcium, magnesium, phosphorus, zinc, iron and manganese were estimated in ash solution using ICP-OES Agilent 5100 VDV according to the US EPA, [18].

2.2.4 Energy value

Calorie values were calculated by procedures outlined by FAO, [19].

Calorie value (kcal/100 g) = (%carbohydrate × 4.1) + (% protein×4.1) + (% fat× 9.1).

2.3 Determination of Some Phytochemical Constituents

2.3.1 Determination of Vitamin C

Vitamin C was determined according methods described by [20].

2.3.2 Determination of phenolic composites

Phenolics were determined by the method described by [21].

2.3.3 Determination of flavonoids

Total flavonoids of crude extract were determined following the method described by [22] modified by [23].

2.3.4 DPPH free radical

The ability of a compound to donate a hydrogen atom was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl2-picrylhydrazyl (DPPH) radical according to a procedure based on [24] with slight modifications by [25].

2.3.5 Determination of amino acids

Amino acids content of MTSP and WF analyzed in using amino acid analyzer. Amino acids were analyzed according methods described by AOAC [26].

2.3.6 Estimation of tryptophan

Tryptophan content of samples was determined calorimetrically according to the method described by [27].

2.3.7 Sensory evaluation

Organoleptic evaluation of bread sticks with and without MTSP were carried out by ten panelists

Table 1. The formulas of bread sticks

Constituents	Control	Formula(1)	Formula(2)	Formula(3)
WF (72%)	100	96	92	88
MTSP (%)	0	4	8	12
Salt (g)	0.5	0.5	0.5	0.5
Yeast (g)	0.6	0.6	0.6	0.6
Sugar (g)	8	8	8	8
Fat(oil) (g)	10	10	10	10

MTSP were replacement the base WF at three several extents (4, 8 and 12%). The control dough was prepared form 100% WF (72%) extraction

to estimate overall acceptability flavor, texture, taste and color. The estimation was prepared using marks from 1 to 10, where, not acceptable (2-3), fair (4-5), very good (6-8), and excellent (9-10) according to methods described by [28].

2.4 Statistical Analysis

Statistical analysis was done using SPSS software (version 15) and Duncan's multiple range tests was used for mean comparison.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of MTSP and WF 72% Extraction Composite (g/100 g on Dry Basis)

The proximate composition of the WF and MTSP shown in Table 2. MTSP were found to contain 25.00% for protein, 25.56% for crude ether extract, 4.55% for ash, 27.61% for available carbohydrates, 27.55% for crude fibre and 440.48 Kcal/100 g for caloric value, respectively. On the contrary, WF were found to contain 11.81% for protein, 0.75% for crude ether extract, 0.45% for ash, 86.13% for available carbohydrates, 0.84% for crude fibre and 398.51 Kcal/100 g for caloric value. The results agree partially with those of [3] showed that MTSP comprise of crude ether extract (29.68%), crude proteins (25.25%) and crude fibre (29.95%). MTSP contain 23.56% for crude protein, 23.12% for ether extract, 4.98 for ash and 30.33% for crude fibre [29]. WF 72% extraction comprised 11.38% crude protein, 0.82% fat, 0.45% ash and 86.79% total carbohydrates [30]. WF 72% extraction contain 0.74% ether extract and 0.83% crude fiber [31].

3.2 Phytochemical Compounds of MTSP and WF (mg/g on Dry Basis)

Table 3 show that phenolic composites for MTSP was higher than those obtained by [15] who explained that phenolic compounds of MTSP is 37.61 mg as Gallic acid / g seeds. It is well known that phenolic compounds, particularly, polyphenols are antimicrobial activity and work as natural antioxidants and oxygen-scavengers [32]. Also, flavonoids content of was 20.65 mg/g. The results agree partially with those of [15] who stated that flavonoid content was 19.51 mg/g in MTSP.

Vitamin C content of MTSP was 4.59 mg/g. while, WF didn't contain vitamin C. Ascorbic acid is essential for the inhibition of scurvy, immune work and potent antioxidant activity. Ascorbic acid has inhibited endothelial dysfunction, apoptosis, cardiovascular disease, anti-inflammatory influences, reduce the hazard of cancer, obesity and reduces the risk of arteriosclerosis [33].

DPPH radical scavenging capacities of ethyl alcohol extracts of MTSP and WF of percentage inhibition of DPPH at 100 ppm are shown in Table 3. The DPPH radical scavenging capacities of MTSP were found to have relatively good free radicals scavenging capacity due to their high DPPH radical inhibition. The oxidative stress caused by free radicals is delayed or even banned by a special class of compounds named as antioxidants. Data available in Table 3 point to that MTSP displayed higher antioxidant activity with DPPH than the WF. These results are in harmony with described by [15] who described that scavenging radical activity shown that MTSP extract of 80% ethyl alcohol extract had 92.76% inhibition.

Table 2. The chemical composition of MTSP and WF (g/100 g on dry basis)

Raw materials	MTSP	WF* 72% extraction
Crude protein%	25.00±0.88	11.81 ±0.11
Crude ether extract%	25.56±0.33	0.75 ±0.06
Ash%	04.55±0.07	0.45 ±0.05
Total carbohydrates%	55.16±0.75	86.97 ±0.39
Available carbohydrates%	27.61±0.25	86.13±0.40
Crude fibre%	27.55±0.55	0.84 ±0.06
Caloric value (kcal/100g)	440.48±2.11	398.51±0.88

MTSP = milk thistle seeds powder; -WF = wheat flour (72%extraction)

Table 3. Phytochemical compounds of MTSP and WF in 80% alcoholic extract (mg/g on dry basis)

Components	Phenolic (mg Gallic/g)	Flavonoids (mg/g)	Inhibition% of DPPH	Vitamin C (mg/g)
MTSP	40.33 ±0.08	20.65 ±0.05	90.88 ±1.45	4.59 ±0.08
WF	0.12 ±0.01	0.04 ±0.00	3.55 ±0.07	ND

3.3 Farinograph Test

Data in Table 4 describe the rheology properties determined by farinograph apparatus for four formulas using WF enriched with MTSP at different extents. From these results it could be noticed that WF enriched with MTSP due to increase the water absorption (%), arrival time(min), development dough(min) and degree of softening (B.U). On the contrary, stability (min) was reduced with incorporation with MTSP. Dough stability is the most important index for dough strength. Dough stability had been attributed to protein poor in sulfhydryl groups, which normally caused softening or degradation action of the dough [34]. This is in contrast with the fact that protein content increased with the increased the substitution level with MTSF, which may be due to the higher fiber content, which destroyed the gluten matrix [13]. Rosell et al., [35] stated that the differences in water absorption is principally caused by numerous hydroxyl groups existing in the fibres and allows more water interaction through hydrogen bonding.

3.4 Mineral Contents of MTSP and WF-MTSP Composite Bread Sticks

Table 5 displayed that content of micro and macro mineral elements of control bread sticks without MTSP and WF-MSTP bread sticks. Bread sticks enriched with MTSP increases the content of iron (Fe), zinc (Zn), manganese (Mn), phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) as compared with control of bread sticks prepare from WF. It is clear from the results that the bread sticks content of mineral elements depends on content of MTSP. The MTSP are comparatively source of indispensable minerals. MTSP contained Fe, Zn, Mn, P, K, Ca and Na were 60.55, 4.50, 3.50, 800.33, 453.85, 935.55 and 50.65 mg／100 g, respectively. Khalil et al. [29] revealed that MTSP contain 880 mg / 100 g for Ca, 750 mg / 100 g for P, 400 mg / 100 g for K, 40 mg / 100 g for Na,65.50 mg / 100 g for Fe, 2.21 mg / 100 g for Mn and 3.76 mg / 100 g for Zn.

3.5 Amino Acids of MTSP and WF 72% Extraction (g /100 g of Protein)

Amino acids comprise of MTSP and WF 72% extraction shown in Table 6. The data explain that crude protein of MTSP contain small quantities of proline (0.60%) and methionine (0.85%). Meanwhile, glycine was 25.50% and threonine were 20.50%. Also, indispensable amino acids in MTSP were higher than WF in exception isoleucine, phenyl alanine, methionine and tryptophan. From the data of Table 6, it could be noticed that, concentrations of amino acids in WF were lower than those present in MTSP. Tyrosine is essential for norepinephrine, adrenalin and dopamine synthesis. Isoleucine is essential for the production of hemoglobin in red blood cells. Leucine has useful influence for bone and tissue wound healing, enhances growth hormone synthesis and skin. Valine and lysine are important for muscle proteins, tryptophan is vital for production of relief depression and neurotransmitter serotonin. Therefore, some indispensable amino acids are presented in herbs and plants foods [36].

Abd Raboh, [15] studied that MTSP contain a high quantities of indispensable amino acids such as threonine, lysine, leucine and poor in tryptophan and methionine.

3.6 The Chemical Composition of Bread Sticks (g／100 g on Dry Weight Basis)

The mean value content of crude ether extract, ash, crude protein, crude fiber and available carbohydrates of the produced bread sticks fortified with 4%, 8% and 12% of MTSP showed in Table 7. Crude fiber, crude protein, ash and ether extract significantly increased in bread sticks enriched with MTSP compared with control. On the contrary, there was a decrease in

Table 4. Farinograph test

Formulas	Water absorption (%)	Arrival time (min)	development Dough (min)	Stability (min)	Degree of softening (B.U)
Control	59.00	0.50	1.00	10.00	50
	±0.40	±0.04	±0.01	±0.25	±1.70
4% MTSP	62.00	1.00	2.00	8.00	90
	±0.50	±0.01	±0.03	±0.20	±1.90
8% MTSP	63.50	2.00	2.50	7.50	120
	±0.50	±0.03	±0.03	±0.10	±2.35
12% MTSP	66.00	2.50	3.00	5.00	130
	±0.65	±0.02	±0.02	±0.20	±2.50

-Means with different letter in the same column are significantly different at LSD at (p ≤ 0.05).
- Each value was an average of three determinations ± standard deviation.
-MTSP milk thistle seeds powder.*

Table 5. Mineral contents of MTSP, WF bread sticks and WF-MTSP composite bread sticks (mg ⁄100 g on dry basis)

Mineral	Fe	Zn	Mn	P	K	Ca	Na
MTSP	60.55	4.50	3.50	800.33	453.85	935.55	50.65
	±01.08	±0.05	±0.07	±2.55	±2.30	±01.98	±1.05
Control	1.80	4.20	0.82	130.50	147.00	16.53	4.85
	±0.05	±0.06	±0.04	±1.50	±01.55	±0.55	±0.06
4% MTSP	4.08	4.22	0.92	156.85	158.50	53.20	6.70
	±0.07	±0.11	±0.05	±1.36	±0.58	±0.58	±0.45
8% MTSP	6.50	4.26	1.03	183.70	170.80	89.90	8.50
	±0.09	±0.04	±0.02	±1.90	±1.30	±0.34	±0.25
12% MTSP	8.80	4.30	1.15	210.50	183.00	126.70	10.40
	±0.04	±0.07	±0.02	±1.95	±1.50	±01.25	±0.22

-Means with different letter in the same column are significantly different at LSD at (p ≤ 0.05).
- Each value was an average of three determinations ± standard deviation.
MTSP = milk thistle seeds powder

available carbohydrates. This may be due to increased amount of crude fiber, ash, total fat and crude protein in MTSP compared with WF. This clearly indicates that MTSP can be an another source of nutritional fiber in bread sticks processing. This study is consistent with hose obtained by [15] cleared that balady bread enriched with MTSP increase in crude ether extract, crude protein, crude fibre and ash. On the contrary, balady breads made from 2.5 and 5% MTSP balady bread decrease in carbohydrates.

3.7 Sensorial Properties of Bread Sticks

Table 8 showed that sensory parameters of bread sticks supplemented with 4, 8 and 12% MTSP. Results of sensorial properties such as overall acceptability, taste, flavor, texture and color are showed in Table 8. The data explained that the formulas enriched with 4, 8 and 12 of MTSP significantly decreased various parameters such as color, taste, flavor, texture and overall acceptability. Bread sticks prepared by partial replacement of WF with MTSP at extent 4, 8 and 12% characterized with good sensorial properties. Sensorial attributes of formulas processed using MTSP at 4% replacement ratio had nearly similar scores compared with those of control. Using of MTSP at extent 12% MTSP led to decrease the scores for sensorial attributes of bread sticks particularly taste of formulas enriched with 12% MTSP was 6.67 and control was 9.00. In general, all formulas enriched with MTSP are acceptable for the sensorial attributions. These results agree with [29] reported that addition of MTSP to WF at extent 5, 10 and 15% of biscuits was acceptable for the sensory evaluation. Shahat, et al. [13] showed that the realized specifications of balady bread not affected by addition MTSP at 9%.

Table 6. Amino acids of MTSP and WF 72% extraction (g /100 g of protein)

Amino acids	MTSP	WF 72%extraction
Essential amino acids(EAA)		
Lysine	6.90	2.55
Isoleucine	5.70	4.00
Leucine	8.60	4.20
Phenyl alanine	2.50	5.23
Tyrosine	ND	1.81
Histidine	2.00	3.98
Valine	5.40	4.36
Theronine	20.50	1.90
Methionine	0.85	1.20
Tryptophan	0.80	1.10
Total (EAA)	53.25	30.33
Non-Essential amino acids(NEAA)		
Aspartic	10.50	5.41
Glutamic	ND	33.11
Proline	0.60	6.70
Serine	4.00	12.6
Glycine	25.50	3.70
Alanine	ND	3.60
Arginine	2.20	2.29
Total (NEAA)	42.80	67.41

MTSP = milk thistle seeds powder; -WF = wheat flour 72% extraction

Table 7. The chemical composition of bread sticks (g/100 g)

Formulas	Crude protein %	Ether extract %	Ash %	Crude fiber %	Available carbohydrates %	Caloric value (Kcal/100g)
Control	9.84^{d} ±0.15	8.80^{d} ±0.22	0.40^{d} ±0.01	0.70^{d} ±0.05	80.26^{d} ±0.85	439.60^{b} ±1.50
4% MTSP	10.30^{c} ±0.12	9.80^{c} ±0.23	0.55^{c} ±0.02	1.60^{c} ±0.04	77.75^{c} ±0.35	440.40^{a} ±1.70
8% MTSP	10.75^{b} ±0.05	10.63^{b} ±0.15	0.67^{b} ±0.02	2.50^{b} ±0.03	75.45^{b} ±0.95	440.47^{a} ±1.60
12% MTSP	11.20^{a} ±0.06	11.43^{a} ±0.11	0.80^{a} ±0.01	3.40^{a} ±0.05	73.17^{a} ±0.46	440.35^{a} ±1.50

-Means with different letter in the same column are significantly different at LSD at ($p \leq 0.05$).
- Each value was an average of three determinations ± standard deviation.
-MTSP milk thistle seeds powder*

Table 8. Sensorial attributes of bread sticks

Samples	Color 10	Taste 10	Flavor 10	Texture 10	Overall acceptability 10
Control	9.50^{a} ±0.58	9.00^{a} ±0.15	9.27^{a} ±0.58	9.17^{a} ±0.76	9.50^{a} ±0.29
4% MTSP	8.50^{ab} ±0.25	8.16^{b} ±0.42	8.67^{b} ±0.49	8.17^{b} ±0.58	8.60^{ab} ±0.40
8% MTSP	7.66^{bc} ±0.76	8.00^{b} ±0.58	8.17^{c} ±0.62	7.33^{c} ±0.26	7.60^{bc} ±0.49
12% MTSP	7.00^{c} ±0.50	6.67^{c} ±0.48	7.33^{d} ±0.38	7.00^{c} ±0.40	7.33^{c} ±0.39

-Means with different letter in the same column are significantly different at LSD at ($p \leq 0.05$).
- Each value was an average of ten determinations ± standard deviation.
-MTSP milk thistle seeds powder*

4. CONCLUSION

Milk thistle seeds enriched bread sticks are new product developed within the framework of bakery products. Bread sticks containing 12% MTSP are highly accepted by the consumers.

As it is good in taste and flavor and have a suitable overall acceptability. MTSP enhanced chemical composition, essential amino acids, and minerals which play an active role in enhancing the nutritious value of bread sticks. MTSP is a main source of several bioactive compounds, particularly flavonoids, polyphenols and vitamin C. In addition, MTSP could be incorporated up to 12% level in the formulation of bread sticks without adversely affecting overall quality and textural characteristics

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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