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Preparation of Bread Supplemented with Milk Thistle Flour and its Effect on Acute Hepatic Damage Caused by Carbon Tetrachloride in Rats

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ABSTRACT

The innovation idea of this research is preparing therapeutic foods for treatment or prevention of liver diseases, which widespread between large sectors of Egyptian population, especially inside the poorest levels. Therefore; balady bread, which pricey available food to this sector, was chosen in our research. Silymarin is bioactive material used for treatments or protection of liver disease people. However, milk thistle plant seeds that heavily grown in Egypt was used as a natural source of silymarin which found to be have 6.81% in defatted milk thistle seeds (MTS). The bread was supplemented by adding milk thistle seed flour (MTSF) at levels of 3, 6 and 9%.The biological effect (using CCL4 intoxicated rats), rheological properties of bread dough and the organoleptic characteristics was evaluated in supplemented bread with MTSF. Silymarin content in bread was detected in tested loaves at different levels of supplements, and compared to the recommended doses either for treatment or protection doses. The results indicated a prospective results on reducing the liver damage which be indicated through ALT, AST, ALP in intoxicated rats blood after the end of experiments. The patients whom suffer from any liver disease could obtain the treatment dose (420 mg/day) when eaten two loaves/day of balady bread contains 3% or one bread have 6 % levels of MTSF. Panelists exhibited good sensory results of the therapeutic bread even at 9% MTSF. The realized specifications of balady bread not affected by addition MTSF at 9%. In general, the use of cheap available natural sources of bioactive material and incorporated to our cheapest foods for protection or treatment of our widespread illness is innovated road to produce low price therapeutic foods available to wide levels of our population.

Key words: Milk thistle, carbon tetrachloride, liver, functional foods, balady bread.

Introduction

The liver regulates many important metabolic functions in the human body, so its injury is associated with distortion of these metabolic functions (Wolf, 1999).It is the key organ of metabolism and excretion, which expose it continuously and variedly to xenobiotics because of its strategic placement in the body (Shaker *et al*., 2010). Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Rao *et al.,* 2006).

The relationship between food and health has an increasing impact on food innovation due to the popularity of the concept of functional food, which designed to improve food consumer's health or meet special health problem (Peressini and Sensidoni, 2009).

In Egypt, as well as the Middle East, the most popular type of bread is a flat (balady bread), circular loaf (1 cm thickness, 10 to 30 cm diameter) consisting of two layers. It is commonly made from high extraction flour (82%) and prepared by a straight dough method (Yaseen *et al.,* 2007).

In developing functional bakery products (such as bread), it is important to develop a product have a physiological effect and palatable by consumers with high quality characteristics (Siro *et al.,* 2008).In Europe, consumption of bread enriched with bioactive compounds is constantly increasing because consumers understand the role of a health-promoting component of such products.

Milk thistle (*Silybummarianum* L. *Gaertn*) is one of the most ancient known herbal medicines, it is an annual or biennial plant belongs to the family Asteraceae (*Compositae*).

The active constituent of milk thistle is silymarin, a mixture of flavonolignans comprised of 4 isomers: silibinin, isosilibinin, silichristin, and silidianin. Most supplements are standardized according to their silibinin (often called silybin) content, the main component of the silymarin (Post-White *et*

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*al.,*2007).This study was aimed to evaluate the effects of different amounts of milk thistle seed flour on rheological and sensorial properties of wheat flour based dough and bread. Moreover evaluate the effect of milk thistle seed diets upon hepatotoxicity induced in albino rats by CCL_4.

Material and Methods

Materials:

Milk thistle seeds (*Silybum marianum*) (MTS) were collected from a farm located in Suqayl village, Ausim city, Giza, Egypt in June 2014. Wheat flour (WF) 82% extraction was purchased from Shahin milling company, Menoufia, Egypt. Salt and bakery yeast were purchased from the local markets.

Silymarin standard was imported from Sigma Chemical Company. All chemicals and reagents of the analytical methods used in the present study was of analytical grade.

Kits for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were obtained from Bio-diagnostic, Dokki, Giza, Egypt.

Standard synthetic diet composed of 12% casein, 8% corn oil, 4% salt mixture, 1% vitamins mixture, 5% fiber (bran) and 70% starch as described by Ismail, (2013).

Thirty five male albino rats weighting between 140-180g were purchased from the Biological Products & Vaccines Holding Company, Helwan Farm.

Methods:

Technological Methods:

Preparation of milk thistle seed flour (MTSF):

Milk thistle seed was milled to pass through 60 mesh sieve. Then defatted with petroleum ether (40-60c°) in a soxhelt apparatus for 16 hours as described by AOAC (2012). The defatted seed powder was de-solventized in air at room temperature (25°C), then reground by blender as mentioned above. The flour was packed in polyethylene bags and stored at -18˚C freezing until used (Abd Raboh, 2012).

Preparation of wheat flour (WF) blends with MTSF:

WF used was divided into four portions. Three portions were individually substituted by 3, 6 and 9 % of MTSF. While the fourth portion was as control (without MTSF addition). The blends were homogenized and stored in airtight containers and kept at 5 –7 ºC until further using. The following Table exhibited the prepared blends of WF and MTSF.

Table: Blends prepared by substitution of wheat flour (WF) with milk thistle seed flour (MTSF)

Wheat flour sample	Wheat flour (WF) (%)	MTSF (%)
Control (wheat flour)	100	-
Wheat flour +3%MTSF	97	3
Wheat flour + 6%MTSF	94	6
Wheat flour + 9%MTSF	91	9

Processing of balady bread:

Balady bread was manufactured from hard flour (82% extraction) using the following formula that described by El-Talawy and Khorshid, (1982) about 500 g hard wheat flour and 500g of prepared blends was mixed individually with 1g table salt (NaCl). Baker's yeast (1.5%).The mixture was mixed till reached to the optimum consistency of the dough. The formed dough was fermented at room temperature for 30 min, then divided into about 150g pieces, rounded, flattened, proofed for 30 min at room temperature and baked at 400-450ºC for 1-2 min.

The bread loaves were allowed to cool at room temperature for 1h before being packed in polyethylene bags and stored at room temperature for further analysis.

Analytical Methods:

Chemical analytical methods:

Determination of silymarin and its fractions:

Silymarin was determined in milk thistle seeds according to (Ismail *et al.,* 2013). About 1 gram of milk thistle seed powder was extracted by petroleum ether for 6 hours and followed by a triple extraction with methanol for 8 hours. The aliquots from each methanol extraction were pooled evaporated at temperature not exceeding 50 °C and the extracted silymarin was obtained as soft yellow powder. The powder was re-dissolved in 10 ml methanol and then measured by HPLC (HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Agilent, USA).).

The breads were sliced (about 1 cm thickness), and dried in an electric convection oven at 40 °C for 24 h. The dried bread was ground and sieved through an 80-mesh screen to obtain bread powder. About 20 grams of bread powder were extracted with methanol for 8 hours. The methanolic extract was evaporated at temperature not exceeding 50°C and extracted silymarin was obtained as a soft yellow powder. The powdered was resolvent in 10 ml methanol and then measured by HPLC.

Effect of baking condition on silymarin content:

The effect of balady bread baking condition (400-450˚C for 1-2 minutes) on silymarin content. The total silymarin content was calculated before baking and compared with it ratio after baking process obtained by HPLC

Bio analytical methods:

Biological evaluation of mixed bread of rats:

Preparation of diets used for rats feeding:

four diets were prepared for the five groups of rats as follows: amount of 82.355 g of the milled control balady bread, 83.855 g of the balady bread containing 3% MTSF, 86.200 g of the balady bread containing 6% MTSF, 87.260 g of the balady bread containing 9% MTSF Each of these amounts were supplemented with varying grams of nutrients including; casein, corn oil, salts, vitamins, fiber (bran) in order to prepare standard synthetic diet for rats as illustrated in Table 1. The ingredients of each diet were added together, properly mixed. The processed diet was packed in labeled polyethylene bags for feeding (Omar, 2009).

Table 1: Supplemented amounts of nutrients added to Balady bread treatments to obtain unit diets like as the formation basal diet.

Nutrients	Basel diet	Control Balady bread (Group 1، 2) (g/82.355g)	Balady bread containing 3% MTSF (Group 3) (g/83.855g)	Balady bread containing 6% MTSF (Group 4) (g/86.200g)	Balady bread containing 9% MTSF (Group 5) (g87.260g)
Protein (casein)	12	2.475	1.777	0.858	0.344
Corn oil	8	7.270	6.912	6.595	6.506
Salt mixture	4	2.318	2.320	1.784	2.113
Fiber (bran)	5	4.583	4.176	3.619	2.811
Vitamin mixture	1	1	1	1	1

Experimental animals and design:

After the adaptation period where the rats fed a standard diets, the 35 male white albino rats were divided into five groups (7 rats / group) as follows:

Group 1: Negative control rats (-ve control) fed on the control bread and injected only with olive oil after an acclimatization period (untoxic rat's liver).

Group 2: Positive control rats (+ve control)fed on the control bread and after an acclimatization period Toxicity was induced by carbon tetrachloride CCL_4 dissolved in olive oil (1ml/kg) weakly for three weeks (El-Beih *et al.,* 2015).

Group 3: This group of rats Fed on the bread containing 3% milk thistle. Toxicity was induced by CCL_4 as positive group.

Group 4: This group of rats Fed on the bread containing 6% milk thistle. Toxicity was induced by CCL_4 as positive group.

Group 5: This group of rats Fed on the bread containing 9% milk thistle. Toxicity was induced by CCL_4 as positive group.

At the end of the experiment, rats were anaesthetized in slight chloroform and blood samples collected into clean tubes, clotting by EDTA and centrifuged at 3000 rpm (Hitachi Centrifuge) for serum separation. The separated serum was used for determinations of liver function enzymes.

Biochemical Analysis:

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to the method of Reitman and Frankel, (1957). Serum alkaline phosphatase (ALP) activity was determined according to the method of Belfield and Goldberg (1971).

Quality evaluation of dough and bread as addition of MTSF:

Organoleptic Evaluation of balady bread:

Balady bread loaves of produced from supplemented WF with MTSF were evaluated organoleptically by 25 coworkers in food science and technology department according to Hussein *et al.,*(2013) using numerical scoring test as described by Ranganna (2011). Each sample was tested for its general appearance (20), separation layer (20), roundness (15), and distribution of crumb (15), crust colour (10), taste (10) and odour (10).

Dough Rheological Properties:

The samples were tested by Barabender Farinogragh (model No. 178507) for determining water absorption and mixing characteristics of dough prepared from the various blends under investigation.

Statistical analysis:

The obtained results were analyzed using analysis of variance (ANOVA) at the 5% level of probability; as reported by Snedecor and Cochran (1994).

Results and Discussion

Silymarin content and it fraction on milk thistle seed:

Silymarin is one of the basic substances used as an element of the adjunctive therapy in the treatment of liver diseases.

Data in Table (2) show total silymarin determined in MTS is 61.80mg/g (6.18%). Our result is fallen inside the range of Halbach and Gorler (1971) they found that silymarin content was ranged from 4 to 6% (based on seed dry weight). But the obtained result was found to be higher than that reported by Khalil, (2008) which was 4.6 % (46 mg/g). while its was less than that reported by Abd Raboh (2012*)* who found that silymarin content of MTS was 70 mg/g, this variation may be related to the varieties, geographic and climatic conditions of growing environment and other agents (Ramawat and Mérillon, 2008). From the same table, total silymarin of MTSF was found to be 88.24 mg/g (8.82%).

Silymarin components were isolated and determined by HPLC. The major component was Silybin 27.29 mg/g (44.17%) of total silymarin followed by silychristin 17.60 mg/g (28.48%), isosilybin 10.73 mg/g (17.36%) and silydianin 6.1716 mg/g (9.98%) as shown in table (2). These results was higher than that reported by Khalil, (2008) who found that silybum marianum contained silybin, silychristin, isosilybin and silydianin as 25.12 (54.6%), 11.09 (24.1%), 6.16 (13.4%) and 3.63 (7.9%) mg/g seed dry weight,

respectively, percentages represent the relative amount of the component to the total. As reported by Gazak *et al.,* (2007) that Silybin compound in between other components of Silymarin, is the most biologically active components used as hepatoprotectant and then it is currently used as therapeutic agent for the treatment of cirrhosis, chronic hepatitis and liver diseases associated with alcohol consumption and environmental toxin exposure and other liver inflammatory diseases according to our results (Table 2), this emphasized that the local varieties of MTS that have more Silybin (27.29 mg/g of silymarin components), and thus, it could have a great effect for liver disease patients. Thereby, it is necessary to incorporate the local MTS with our food products to produce innovated therapeutic food valid to treatment liver disease patients that excessively distributed among our population in Egypt (National Liver Institute, 2016).

Table 2: Silymarin content and its constituents (mg/g) in milk thistle seed powder and flour

Constituents	MTS powder (mg/g)	MTS flour (mg/g)
Silybin	27.295	38.978
Silychristin	17.602	25.135
Isosilybin	10.731	15.324
Silydianin	6.172	8.813
Total silymarin (mg/g)	61.80	88.24
%	6.1	8.82

The level of silymarin in bread supplemented by milk thistle flour and effect of baking temperature on silymarin content:

Data in Table (3) show that the balady bread prepared with 3, 6 and 9 % MTSF contained 2.46, 4.93 and 7.40mg silymarin / g bread, respectively.

Consequently, the total silymarin in whole balady bread (120g) substituted by different levels of MTSF was found to be 295.2mg silymarin /loaf contained 3% MTSF, 591.5mg silymarin / loaf contained 6% MTSF and 888mg silymarin / loaf contained 9% MTSF.

The addition of milk thistle seed flour to bread led to creating bread rich in silymarin (therapeutic bread) suitable for liver disease treatments or at least for liver protection.

Table 3: Silymarin content in balady bread:

Balady bread samples	Total silymarin (mg/g bread)	Total silymarin in whole bread (mg / loaf)
Bread containing 3% MTSF	2.46	295.2
Bread containing 6% MTSF	4.93	591.5
Bread containing 9% MTSF	7.40	888

As recommended by Brown, (1996), Takao Co., (1996), the total daily dose of silymarin required for the treatments of liver disease (hepatitis, cirrhosis, toxin damage), is 420 mg divided at three doses/day along the period ranged between 4 weeks: 9 months. But, when silymarin used for preventive purposes (as protective agent from liver diseases) this requires a doses ranged between 210: 280 mg Silymarin/day, as also recommended by the previous authors. Such quantities of silymarin doses meeting the consumption of 4.75 and 3.17 g of MTSF as nutritional supplement, respectively for treatment and protective agents (Table 2). According to our results, such silymarin doses could be obtained from consuming two loaves of bread supplemented with 3% MTSF per day (meeting 591mg of silymarin, Table 4) this was calculated depending on the average weight of balady loaf which normally being 120 g, as prepared in our study. However, as clearly shown in Table 4, if MTSF wanted to use to protect the liver from any disease, this could be enough by consumption one loaf containing 3% MTSF per day that could supply the silymarin dose for liver protection (295.5 mg/day). Consequently, the prepared bread containing 6 and 9% MTSF surely achieving both prevention and therapeutic doses.

Thus, addition of MTSF to our foods (as a source of silymarin) could properly achieve our aim of producing therapeutic foods suitable for the nutrition of liver disease patients that widely diffusion between our population, especially between the poorest levels. Thereby, to achieve our ideal of improving or reducing the percent of liver disease states in our country, we chosen balady bread in our study as a more food production that widely available and much consumed by poorest population that relatively suffering from a problem in their livers.

According the Human studies that have shown a silymarin to be generally safe and have not side effects (Abenavoli, 2010). As reported, the typical adult dosage tolerance for silymarin is 240: 900 mg/day distributed in two or three divided doses. It could be recommended that the person whom suffering liver diseases must be consumed balady breads containing 9% MTSF which supply 2664 mg silymarin if eaten

3 loaves/day. However, at doses more than 1500 mg/ day, silymarin may produce a laxative effect due to increased bile flow and secretion. Mild allergic reactions have also been noted, but neither of these side effects was severe enough to discontinue treatment Luper, (1998).

Table 4: Silymarin intakes from balady bread made of different substitution levels of milk thistle seed flour at different daily consumption.

Balady bread samples	Silymarin content obtained from		
	1 loaf /day	2 loafs /day	3 loafs /day
Bread containing 3% MTSF	295.5	591	873
Bread containing 6% MTSF	591.6	1183.2	1774.8
Bread containing 9% MTSF	888	1776	2664
Achieve Treatment (therapeutic) dose (420 mg)/day	- ve	+ve	+ve
Achieve Preventive dose (280 mg)/day	+ve	+ve	+ve

By comparing the silymarin content of balady bread before and after baking, it could be observed (Table 5) that at elevated temperature during baking (~ 400˚C) caused a slight reduction in silymarin content before and after baking ranged between 92.97: 93.22%. Although, as reported by Duan, (2005) the extracted pure silymarin is affected to be destruction by high heat, however, as we predicted, the using of silymarin as it natural form inside the MT seeds, as used in present work, may be give the silymarin to be have more stability to high heat. This may explain the discrepancies between our results and that reported by Duan, (2005) and others. Also, the retention of most silymarin quantities (92.97%) after baking temperature may be due to exposure time of baking temperature which be too little (~ 2 minutes) that did not enough to have a large influence on silymarin before and after baking.

Table 5: effect of baking conditions on silymarin content

Balady bread samples	Silymarin content before baking (mg/g)	Silymarin content after baking (mg/g)
Bread containing 3% MTSF	2.646	2.46
Bread containing 6% MTSF	5.292	4.93
Bread containing 9% MTSF	7.938	7.4

The protective Effect of therapeutic bread on rat's liver intoxicated by CCl4:

Table (6) shows that intoxicated rats with once dose of CCl4 (1 ml/kg body weight weakly) led to the development of severe hepatic injury when compared to the rats (-ve control). But, the intoxicated rats that fed on diets containing 3, 6 % or 9% MTSF showed an improvement on their livers. This was obviously shown in the values of AST, ALT and ALP which significantly decreased in animals fed on MTS diets in comparison with control group. These results in agreement with Raja *et al.,* (2007) who found a significant rise in levels of SGOT, SGPT and ALP by CCL_4. But, plant extract and standard drug (silymarin) significantly decreased enzymes levels. The stabilization of these enzymes by crude extract is a clear indication of the improvement of the functional status of the liver. Also, Shaker *et al.,*(2010) studied the effect of extracts from *Silybum marianum* on liver damage caused by CCL_4. Their activity was compared with standard hepatic drug hepaticum for 10 days. The extracts for *Silybum marianum* showed the most significantly decrease in the liver enzymes (GOT, GPT and ALP).

Table 6: Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in control and different treated groups of male albino rats:

Rat Groups	AST (*U/l*)	ALT (*U/l*)	ALP (*IU/l*)
-ve control (control diet)	91 ^{e}	20 ^{c}	10 ^{b}
+ve control (control diet + CCL4)	287 ^{a}	56 ^{a}	18 ^{a}
Bread 3% MTSF	165 ^{b}	23^{b}	18 ^{a}
Bread 6% MTSF	105 ^{d}	18 ^{d}	8 ^{c}
Bread 9% MTSF	156 ^{c}	21^{c}	9.8^{b}

The quality and acceptability of bread as addition of MTSF:

Sensory evaluation of balady bread:

The effects of wheat flour substitution with different level of milk thistle seed flour (MTSF) on the sensory characteristics of balady bread are presented in Table (4).

From the results presented in Table (7) it could be noticed that balady bread containing (3, 6 and 9%) MTSF exhibited satisfactory organoleptic characteristics. It could be noticed that the increment in substitution level of MTSF led to decrement in the mean value of appearance score of the produced bread.

This trend was also observed when color of crust was evaluated, since the bread containing 3 % MTSF (7.75) was nearly similar to the control sample (8.67) followed by bread containing 9 % MTSF (7.67). While the lowest value of bread color was observed for sample containing 6 % MTSF treatment (7.25).

It is interesting to note most panelists mentioned that the balady bread had milk thistle flour gives sense balthabb in tongue during chewing. The sense was increased as the substitution ratio increased. This may be related to the presence of some seed coat of the milk thistle Seeds that contain a high proportion of fiber (lignin). These results are in agreement with Abd Raboh (2012) who found that the appearance of balady bread fabricated with different levels of milk thistle seed had darken color, and gives sense balthabb in tongue during chewing.

The results in Table (7) were not exhibiting any significant difference in crumb distribution and the separation of layers. Also, Table 7 shows that sample with the lowest amount of MTSF (3%) showed the highest value of overall acceptability.

Table 7: Effect of wheat flour substitution with different levels of milk thistle seed flour (MTSF) on organoleptic characteristics of balady bread.

Treatments	Organoleptic characteristics							
	Appearance (20)	Color of crust (10)	Taste (10)	Odor (10)	Roundness (15)	Crumb distribution (15)	Separation of layer (20)	Total score (100)
Control	19.00 ^{a}	8.67 ^{a}	9.00 ^{a}	8.75 ^{a}	12.17 ^{a}	13.00 ^{a}	17.33 ^{a}	87.92 ^{a}
Bread + 3% MTSF	16.67 ^{ab}	7.75 ^{ab}	8.08 ^{b}	8.25 ^{a}	12.75 ^{a}	12.42 ^{a}	17.50 ^{a}	83.42 ^{ab}
Bread + 6% MTSF	15.67 ^{b}	7.25 ^{b}	7.50 ^{b}	7.67 ^{a}	12.75 ^{a}	12.08 ^{a}	17.00 ^{a}	79.92 ^{b}
Bread + 9% MTSF	15.00 ^{b}	7.67 ^{ab}	7.50 ^{b}	8.16 ^{a}	13.83 ^{a}	12.83 ^{a}	18.16 ^{a}	83.15 ^{ab}
L.S.D. at 5%	2.978	1.303	1.009	1.005	2.011	1.246	1.951	6.757

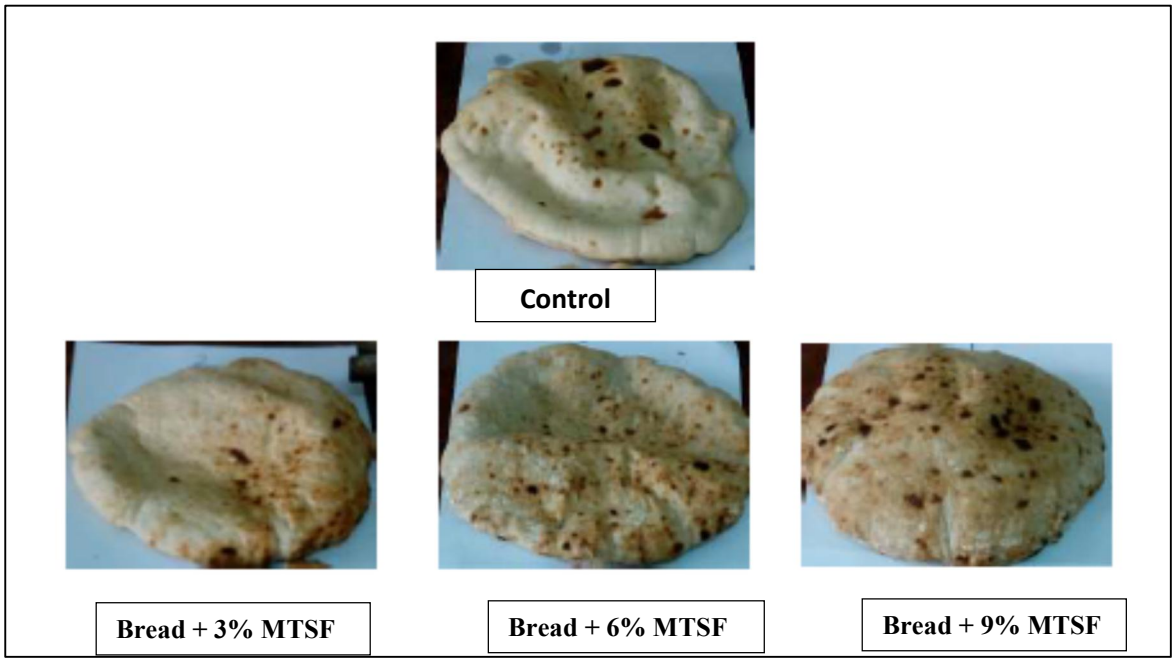

Fig. 1: Balady bread substituted with milk thistle seed flour (MTSF)

Rheological Properties of Flour Dough Samples Used:

The rheological properties of dough prepared from wheat flour and the different blends were evaluated by Farinogragh. Farinogragh properties of wheat flour dough as affected by substitution at different levels of MTSF, the water absorption was increased as the substitution level increased in milk thistle seed flour blends as compared with control treatment, the highest water absorption level was observed for 9 % MTSF treatment where the lowest value of water absorption was observed for the control treatment. This is may due to the high fiber content of milk thistle seed flour which agree with the foundation of Rosell *et*

al.,(2001) who reported that hydroxyl groups, which exist in the fiber structure, allows more water interaction through hydrogen bonding.

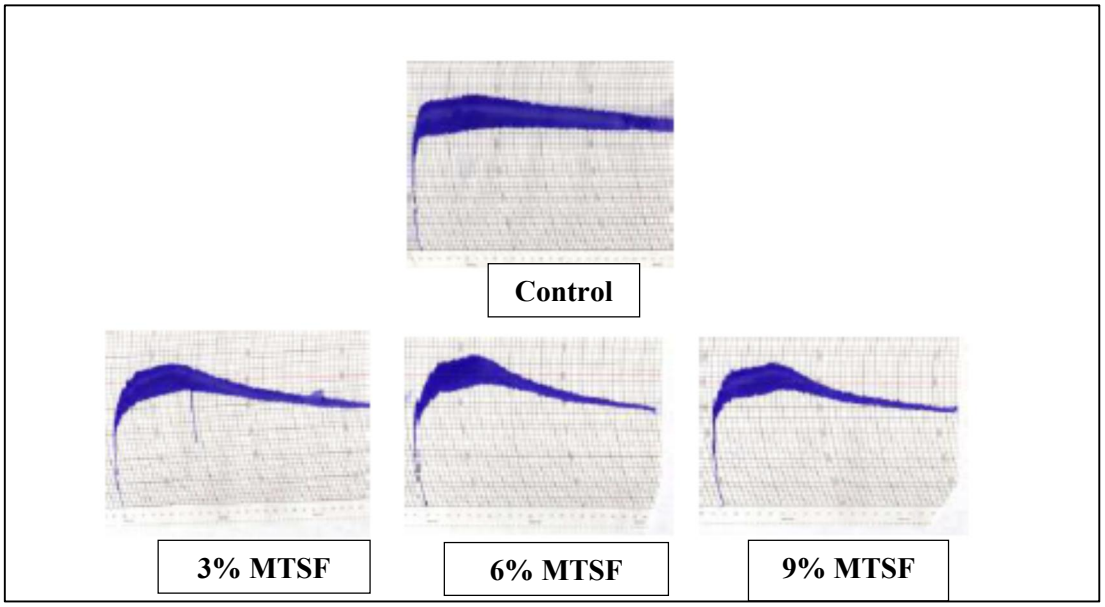

Fig. 2: Effect of substituted wheat flour with 3, 6 and 9 % milk thistle seed flour on farinogragh parameters.

Results presented in Table (8) Show that the arrival times of control and 9% MTSF containing treatments were 2.0min, while the highest value of arrival time (2.5min) detected of samples contained 3 and 6% MTSF. Concerning the dough development time, the addition of milk thistle seed flour (MTSF) increased the development time of the dough.

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 (Ismail, 2007). As shown in data presented in table (8). It could be observed that dough stability significantly decreased with the increase of milk thistle seed powder flour addition level, since the dough stability (min) of MTSF va1ues were 10, 8 and 7.5 min for the treatments containing 3,6 and 9 % MTSF respectively, these results were lower than that obtained for the control sample which was 22 min. 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.

Table 8: Farinogragh properties of balady bread dough substituted with different concentrations of milk thistle seed flour (MTSF)

Treatments	Farinogragh properties					
	Water absorption (%)	Arrival time (min)	Dough development time (min)	Stability (min)	Mix tolerance index (BU)	Weakening (BU)
Control	55	2	4.5	22	30	70
3% MTSF	57.5	2.5	6	10	50	110
6% MTSF	58.5	2.5	5	8	70	120
9% MTSF	60.5	2.0	5.5	7.5	80	110

From the same table, it could be observed that the mixing tolerance index and degree of weakening increased with increasing the substitution level of milk thistle seed flour as compared to control sample, this result might be due to the high fiber and fat contents, which weakened the dough.

Conclusion

The results of this study suggest that the natural source of silymarin from milk thistle seed flour may be blended with wheat flour at levels (0, 3, 6 and 9%) to obtain silymarin enriched balady bread and the sample containing 3% milk thistle seed flour was the best in terms of rheological characteristics and

general acceptance by consumers and consumption of milk thistle -based diet maintains the integrity of the liver and protects it against damage caused by carbon-tetrachloride induced hepatotoxicity.

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