Egypt. J. Chem. **Vol. 64,** No. 4 pp. 1897 - 1909 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Comparative Evaluation of Some By-products substitution in Snacks: The influence on Volatiles Profile and Lessening Aflatoxins Formation

Ahmed M. S. Hussein[a], Gamil El Sayed Ibrahim[b*] Ahmed Noah Badr[c],

[a]: Food Technology Dept., National Research Centre, Dokki 12622, Cairo, Egypt.

[b]: Chemistry of Aroma and Flavor Dept., National Research Centre, Dokki 12622, Cairo, Egypt.

[c]: Food Toxicology and Contaminants Dept., National Research Centre, Dokki 12622, Cairo, Egypt.

Abstract

Snacks are routine light-food, consumed between meals by children or adults. It is important to enhance their safety properties and shelf life, without losing the acceptability. This investigation aimed to achieve that, using a modified rice straw (MRS), stabilized rice bran (SRB), and barley bran (BB) to substitute wheat flour (WF) in functional snacks. The microbiological-safety impact of substituted materials and their aflatoxins-prevention were evaluated. Antifungal properties against toxigenic fungi were arranged to descend as MRS>SRB> BB. Organoleptic characteristics were significantly decreased at a higher SRB and MRS substitution-values. The best snacks-acceptability was recorded for a 10% substitution ratio. Therefore, volatiles content was identified using GC-MS analysis at 10 % substituted snack. The most predominant volatiles were 2,3-butanedione, 3-methyl butanal, and 2-methyl butanal by concentrations of 26.84%, 11.48%, and 7.94%, respectively in control snacks. These volatiles activities are explaining degradation of aflatoxin-secretion.

Keywords: Bran; snacks; volatile compounds; aflatoxins.

1. Introduction

Rice straw is a plant-remains that resulted after the rice post-harvested, where recently it was represented as a real problem for the environment. Rice straw considered rich in phytochemicals and active component [1]. It is a healthy source of bioactive phytochemicals like γ-oryzanol, tocopherols, tocotrienols, and hypoallergenic protein [2]. These materials were not adequate for directly consumption without a pre-treatment [1]. The stabilization commonly used to prolong the bran's shelf life, enabling it to integrate back into the diet.

Implementation of rice byproducts to enhance numerous bakery products successfully carried out without significantly changing of their functional or textural characteristics. The fortification could influence both the physical and chemical characteristics of the flour. Numerous bran types were utilized from wheat, oat, corn, and barley to produce high-fiber bakery products [3].

Flavor is an important quality attributes of baked products, it reflects the consumer acceptability and preference. Bakery flavors were formed mainly during production as result of enzyme reactions, fermentation, or thermal treatment. Recently, in baked products about 300 flavor compounds were identified. Several factors and reactions are responsible for the flavor formation in bakery products, like fermentation, lipid oxidation, Millard caramelization, and thermal reactions. The resultant compounds of these reactions could play vital or safety roles depending on their activities [4].

Backed products were faced by various threatens connected to their safety, in both their raw materials or during the storage. These hazards are including toxigenic fungal growth and their related mycotoxins.

*Corresponding author e-mail: E-mail: gamilemad2000@gmail.com.; (Gamil El Sayed Ibrahim).
Receive Date: 07 December 2020, Revise Date: 24 December 2020, Accept Date: 27 December 2020
DOI: 10.21608/EJCHEM.2020.52472.3086

Aflatoxins (AFs) engage with great hazards in food commodities, which represents a risk through their consumption [5]. AFs became a significant factor of food refusal [6], which requested a necessity of search regarding a novel application targeted to suppress fungal growth and achieve mycotoxin reduction. The application of natural substances for fungal inhibition and AFs reduction, also reflects nutritional and health benefits [5].

This investigation was aimed to evaluate the antifungal and aflatoxigenic properties that suppress the AFs-production, which were contained of the MRS, SRB, and BB extracts. Besides, the estimation of their effect on sensory and volatile constituents of fortified snacks. The current vision pointed to the limitation of toxigenic contamination and cross-contaminations of backed products through their natural-components fortification. These components practice and realizing a preservative function in backed products, besides their involvement in the flavoring ingredients.

Materials and methods

Materials

The WF was obtained from Cairo Mill Company, MRS and SRB were obtained from Kafr El-Sheikh Rice Milling, while the BB was obtained from the Spices-dealer shop. All chemicals used in this study are of analytical grade from Sigma-Aldrich Co.

Methods

Rice straw and bran preparation

The MRS was prepared according to the method described by Mohamed et al. [7]. The SRB was stabilized immediately at 110°C/10 min using a heating-air oven.

Evaluation the physicochemical properties of raw materials

Moisture, ash, fiber, protein, and fat content of raw materials were determined using the same methodologies described in the AACC [8]. FIWE Raw Fiber Extractor (VELP) unit were used to determine hemicellulose, cellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). The changes in Hunter color parameter (L*, a* and b*) of raw materials and different snacks were followed up using the Tristimulus Color Analyzer (Hunter, Lab Scan XE, Reston, Virginia) with standard white tile.

Preparation of the extracts

The extracts of the MRS, SRB, and BB were prepared using a closed system unit connected by a pressure pump. The cycle of the extraction was adjusted at 40°C/1 bar/ 60 min. At the end of extraction-cycle, the extracted materials were filtrated where filtrate and retentive extract were collected separately. The filtrate was dried at hot-air oven (40 °C) until obtained a constant weight, and the retentive extract were lyophilized for further evaluation. For antifungal evaluations, the lyophilized extract (100 mg) of each material individually was dissolved using 0.5 mL dimethylsulphoxide (DMSO) for application against toxigenic fungi and antimicrobial evaluation.

Determination of total phenolic content and antioxidant activity

Total phenolics (TPs) were carried out using the Folin-Ciocalteu procedure with some modification according to Singleton et al. [9]. The absorbance was measured at 760nm and the results were expressed as milligrams Gallic acid equivalent (mg GAE)/g of sample.

The antioxidant activity of MRS, BB, and SRB was evaluated using three assays ABTS*, DPPH, and FRAP as the same methodology previously described [5]. The standard curve was prepared using Trolox and the results were expressed as mM Trolox equivalents (mM TE/g sample).

Snacks manufacturing and sensory evaluation

Snacks were manufactured by the methodology described in Wójtowicz et al. [10] with modifications. The MRS, SRB, and BB individually were added to snacks-dough at 5%, 10%, 15%, and 20% replacement of the WF in a special methodology. It was taken into account that the pressed warm-water firstly used to extract each amount material used for the replacement process, and then the filtrate and extract of each amount were mixed separately into the dough ingredients. Snacks were estimated for their sensory properties where the test-panels was performed according to the method described in AACC [8].

Volatile compound determination

The volatile contents of prepared snacks at wheat flour replacing levels of 10% by BB, MRS, and SRB were estimated as stated by Agilent technologies (Palo Alto, CA) HS autosampler (7697 A) was used to monitor the static HS quantitation of volatiles. Samples (about 0.5 g) were equilibrated for 50 min at 40 °C prior to analysis. The settings of the HSS 7697 A were 5 s for pressurization, equilibration, and filling and 2 min for injection. The HS loop (3 mL) temperature was set at 90°C. High-purity helium, filtered through moisture and oxygen traps (Hewlett-Packard), was used for vial pressurization, and an HSS sampler carrier gas at a flow rate of 17.5 mL/min was measured at the splitter outlet.

GC–MS analysis

Analyses were performed on Agilent 7890 GC coupled to a 5977 MS detector. Manual tuning of the MS with perfluorotributylamine was used to adjust relative abundance for *m/z* 69, 219, and 502. The MS was run in the scan mode (*m/z* range from 33 to 400 with a threshold of 100 and a sampling rate of 3 scans/s). Ultrapure helium was passed through moisture and oxygen traps and was used as the carrier gas. The following GC operating conditions were used: a silica capillary column HP Innowax cross-linked polyethylene glycol adipate (60 m × 0.25-mm × 0.25-μm film thickness); a flow rate of 1 mL/min at 40°C; a split ratio of 1:10; the injection port set at 250°C and the interface line to the MS at 230°C; and the electron energy and electron multiplier voltage at 70 eV and 1647 V. The oven temperature was held for 5 min at 35 °C, then increased by 5 °C/min to 50 °C and held in isothermal conditions for 5 min, then raised to 210 °C at 5.5 °C/min, and finally held constant at 210 °C for 5 min.

Volatile compounds identification

The linear retention index (RI) values for unknowns were determined based on retention time data obtained by analyzing a series of normal alkanes (C_6-C_{22}). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory [11].

Rheological properties

Rheological properties of dough were evaluated using Farinograph and Extensograph apparatus according to the method described in AACC [8]. The Farinograph was utilized for estimating of arrival time, development time, stability time, weakening, and mixing tolerance index of snacks-dough, the parameters of water absorption, while the Extensograph was utilized to measure the extensibility, the resistance to Extension, the extension ratio, and the energy. These parameters were reflected the changes that happened due to the substitution process, and was gave indication for the acceptability of backed product.

Determination of antimicrobial potency assays

The spot-on-lawn technique was utilized for the evaluation of antimicrobial activity for extracts, using a spore solution of each microbial strain. The antifungal potency expressed as arbitrary units per milliliter (AU/mL) defined as the reciprocal of the highest dilution in which fungi were inhibited [6].

Determination of antifungal activity

The growth inhibition test was used to determine the antifungal activity of the BB, MRS, and SRB against toxigenic strains by different assays. Firstly, extracts activity estimated using minimal fungicidal concentration assay as previously described [12].

Inhibition of aflatoxins (AFs) secretion in liquid media

A strain of *A. parasiticus* ITEM 11, which previously reported to produce AFs was applied to estimate the reduction effect in the presence of BB, MRS, and SRB against the control. After 6 days of incubation (25°C), AFs were extracted from the media, evaluated for treatment, and control using the HPLC apparatus according to the method and conditions that were previously described by Shehata et al. [6].

Determination of AFs by HPLC

AFs standard received as dry films, which were prepared for HPLC evaluation. One hundred microliters of the samples were injected into the HPLC (Water 1100); AFs were estimated according to the condition described by Badr and Naeem [5].

Determination of Snacks resistance of fungal contamination

A contamination-stimulate experiment was done to evaluate the resistance of substituted snacks against active toxigenic fungi (*A. parasiticus* ITEM 11). Using sealed sterilize plastic bags, three groups of 10% substitutional snacks using MRS, SRB, BB, plus two control groups were packed. The control groups were negative control and positive control, which positive was prepared using 1000-ppm sodium propionate as a preservative. The negative control was utilized as an indicator of the neutral shelf life without using preservatives, where positive control was indicated the shelf life of chemically preserved snacks. All bags were sealed completely after additional of cotton swap loaded by 0.5mL spore-suspension ($0.4x10^2$ CFU/mL). The increment of the contamination rate was reflected in the weak substitutional effect. The results were expressed as a fungal CFU/g of snack.

Statistical evaluation

The obtained results were evaluated statistically using analysis of variance (One way ANOVA analysis) using SPSS 16.0 as reported by Mc-Clave and Benson [13].

Results and discussion

Physicochemical properties of the BB, MRS, and SRB

The present study explores the replacement impact of the WF in snacks premix with BB, MRS, and SRB at levels elevated up to 20%. The chemical composition of the MRS recorded the lowest contents of moisture, protein, and fats, and the highest content of the crude, fiber fractions, and ash (Table 1). The SRB manifested a lower carbohydrate content. A higher MRS-fiber contents, crude or fractions, could participate in the AFs reduction through their ability for binding unfavorable and undesirable food contaminants. Regarding the color properties of the raw material, a variation was recorded between the MRS, SRB, BB, and WF that affect snacks-color. This effect were varied in parallel with the replacement ratio.

Table 1. Chemical composition, fiber fractions, and color attribute of raw materials

	WF	BB	MRS	SRB
Chemical composition				
Moisture (%)	12.88 ±0.14	13.85±0.22	4.40±0.03	10.55±0.15
Protein (%)	11.25 ±0.19	12.52±0.17	3.47±0.02	14.16±0.19
Fat (%)	2.41±0.06	2.16±0.03	0.11±0.17	8.12±0.05
Ash (%)	1.68±0.17	5.62±0.17	22.96±0.12	7.16±0.19
Carbohydrates (%)	68.7±1.62	53.5±0.86	39.81±0.45	32.83±1.03
Crude fiber (%)	3.08±0.13	12.35±0.19	29.25±0.16	27.18±0.15
Fiber Fractions				
NDF	5.72±1.05	40.82±2.13	74.39±2.79	32.68±1.66
ADF	1.56±0.41	11.85±1.08	50.30±1.52	9.43±1.13
ADL	0.35±0.05	2.40±0.12	10.71±0.83	2.00±0.1
Lignin	0.33±0.08	1.25±0.14	5.64±0.61	1.10±0.06
Cellulose	1.23±0.17	9.51±1.28	24.09±1.56	7.20±1.19
Color Parameters				
L*	84.18±0.85	70.70±1.62	65.22±1.14	73.92±1.35
a*	1.42±0.01	6.13±0.12	8.12±0.13	4.76±0.19
b*	15.63±0.10	20.12±0.14	39.94±0.24	17.45±0.22
Total phenolics (mg GAE/g) and the AA (mM TE/g)				
TP	0.418	3.779	7.363	4.800
DPPH	0.838	4.434	6.196	4.057
ABTS	0.851	4.539	6.041	4.169
FRAP	0.651	4.043	6.274	4.174

Values represented in mean ± SD; and calculated based on the dry weight, TP: total phenolic content; AA: antioxidant activity.
WF: wheat flour, BB: Barley bran, MRS: Modified rice straw, SRB: Stabilized Rice bran, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent Lignin.

Crude and fiber fractions content could represent the main defense against food contamination and harmful components. Early studies classified the fiber function to have functionality in mycotoxin binding. Additionally, [13] evaluated the micronized dietary fiber binding effectiveness on mycotoxin activity in the biological systems. Their result indicated a high effective impact of fibers by mycotoxin sequestration inside the fiber-net. The MRS (the highest fiber fractions content) could serve in mycotoxin contamination reduction, specially its contents of lignin and cellulose fractions [14].

Total phenolic content and antioxidant activity

Total phenolics (TP) showed convergent values for the MRS, BB, and SRB, which was raised nine times than their value in the WF. The MRS recorded the highest TP content (7.363 mg GAE/g), while BB and SRB recorded 3.779 and 4.800 mg GAE/g, respectively. These results is recommended its bioactive functionality in food application. The insertion of these MRS, BB, and SRB elevate the dough content of phenolic compounds so elevates its antioxidant activity. In previous studies, TP-content recorded a positive impact on food-hazard elimination, which include toxigenic fungi inhibition. AFs-secretion reduction, and mycotoxin degradation [5,7].

Similarly, the antioxidant activity (AA) was determined using three different assays. For SRB; the AA values were recorded at 4.169 and 4.174 mM TE/g in ABTS and FRAP assays, respectively. For BB, it was 4.539 and 4.043 mMTE/g in ABTS and FRAP assays, respectively. While MRS showed values of 6.041 and 6.274 mM TE/g in ABTS and FRAP assays, respectively. This could give evidence for resistance properties gained against microorganism contamination in food. Higher AA associated with oxidative stress, which earlier reported cause a breakup food harmful like mycotoxins [15-16]. The higher AA supports the degrading impact of AFs hazards. It also could delay toxigenic fungi excretion of mycotoxin as result of oxidative stress [5].

Sensory evaluation of the snacks

Table 2. Sensory attributes of snacks incorporated with various levels of barley bran, modified rice straw and stabilized rice bran

Samples	Color (20)	Crispness (20)	Taste (20)	Flavor (20)	Appearance (20)	OAA (100)
Control	19.49^{a}±1.45	19.93^{a}±1.13	19.49^{a}±1.39	19.35^{a}±0.95	18.58^{a}±0.1.13	96.30^{a}±2.13
BB (%)						
5	19.38^{a}±1.11	19.55^{a}±1.22	19.30^{a}±1.61	19.15^{a}±0.76	18.48^{a}±1.35	93.40^{a}±2.11
10	19.22^{a}±1.16	19.42^{a}±1.27	19.11^{a}±1.70	18.77^{a}±0.83	18.36^{a}±1.40	92.00^{a,b}±2.55
15	18.78^{a}±1.19	19.32^{a}±1.15	18.14^{a}±1.63	18.75^{a}±0.71	18.22^{a}±1.50	90.50^{b}±3.12
20	18.46^{a}±1.22	19.02^{a}±1.17	18.82^{a}±1.29	18.42^{a,b}±0.69	18.04^{a}±1.60	89.60^{b}±2.19
MRS (%)						
5	17.44^{b}±1.55	17.74^{b}±1.21	17.59^{b}±1.38	18.16^{b}±0.91	16.96^{b}±1.70	87.30^{c}±2.50
10	16.78^{b}±1.32	17.16^{b}±1.23	17.32^{b}±1.46	18.21^{b}±0.96	16.42^{b}±1.76	85.60^{c}±2.33
15	10.60^{d}±1.28	10.10^{e}±1.27	12.25^{d}±1.33	15.80^{d}±0.84	10.24^{d}±1.80	58.99^{f}±1.90
20	10.34^{d}±1.17	10.26^{e}±1.23	11.82^{d}±1.50	15.08^{d}±0.66	10.51^{d}±2.01	58.01^{f}±2.10
SRB (%)						
5	15.30^{c}±1.19	15.79^{c}±1.25	16.66^{b,c}±1.40	17.90^{c}±0.77	15.51^{c}±1.16	81.10^{d}±3.12
10	14.94^{c}±1.26	15.72^{c}±1.35	14.52^{c}±1.43	17.55^{c}±0.89	14.61^{c}±1.33	74.80^{e}±2.44
15	11.71^{d}±1.39	11.63^{d}±1.50	10.37^{d}±1.49	12.64^{e}±0.62	11.09^{d}±1.39	57.44^{f}±2.65
20	9.52^{e}±1.43	11.43^{d}±1.63	10.14^{d}±1.55	11.12^{e}±0.96	10.91^{d} ±1.60	53.12^{g}±2.70
LSD 0.05	1.192	1.092	1.355	0.925	1.245	3.95

The wheat flour substitutions were applied (at 5, 10, 15, and 20%), for each material (as mix of extracted powder and its extract content)., The data with same letter in column are not significant (at p=0.05)., OAA: Overall acceptability, WF: wheat flour, BB: barley bran, SRB: stabilized rice bran, MRS: modified rice bran

The mean value of overall sensory quality of snacks showed a decreasing trend with a proportionate increase of SRB, BB and MRS supplementation (Table 2). The color score of the sample prepared by replacing wheat flour with barley bran is not significantly different ($P \leq 0.05$) and was comparable to the control treatment.

The browning color of bakery products like bread, snacks might be due to starch caramelization, dextrinization, or the non-enzymatic reaction (Maillard reaction). Taste is the most important sensory attribute for the acceptance of the product. Sensory evaluation of snacks showed a significant decrease with an increase in the level of SRB and MRS; while the least decrease was in the BB compared to the control sample (Table 2). The reductions obtained were comparable to those previously reported with added cereal bran and different fiber sources.

Similar to this study by Carroll [17] that reported a high level of rice bran in muffins affects overall appearance, volume, taste, and texture. The mean score of crispness decreased from 19.02 to 11.43 with the level of substitution 20% of the BB and SRB, respectively. The treatment barley bran had the highest scores for the entire sensory attributes than other treatments. Hence, it was concluded from the results that replacing wheat flour with barley BB, MRS and SRB at 10 percent is more suitable for the production of barley bran supplemented snacks. Therefore, this treatment was subjected to evaluate the volatile constitutes using GC and GC-MS.

Effect of the BB, MRS, and SRB on volatile compounds of snacks

The determination of volatile constituents in snacks treated at 10 % of of MRS, SRB or BB were carried out by dynamic headspace GC-MS analyses and identified volatile compounds are given in (Table 3**).** The most predominant volatiles were 2,3-butanedione, 3-methyl butanal, and 2-methyl butanal by concentrations of 26.84%, 11.48%, and 7.94%, respectively in control snacks (Table 3). The amelioration was recorded for the compounds that previously known with antifungal potency. These compounds were included 2- ethylfuran, 2-butylfuran, 2-pentylfuran, 2,3-butanedione, α–terpinene, α–terpinolene, 2-heptanone, heptanal, and ethyl heptanoate [18]. Other aldehydes and ketones like 2-methyl butanal (7.94%); 3-methyl butanal (11.48%); 3-hydroxy-2-butanone, were achieved an antimicrobial properties.

Regarding the 32 identified volatile compounds there are several pathways for its formation. For example, 3-methylbutanal is a Strecker aldehyde originating from isoleucine and produced during baking [19], 1-penten-3-ol from the lipoxygenase activity, and octanal by lipid oxidation have been reported as important contributors to wheat bread crumb aroma with positive correlation with the final aroma of bread [20]. The high concentrations of the 2,3-butanedione; 3-methyl butanal, and 2-methyl butanal in snacks prepared using the BB in parallel with the good acceptability and high scores of sensory evaluation. In our previous study [1] on the evaluation of several bran on volatile compounds of Egyptian bread we found that the main volatile compounds were alcohols. However, the main volatile constituents in the current study on snacks were aldehydes such as 2-

methyl butanal and 3-methyl butanal and ketones like 2,3-butanedione. These differences may be due to the variation in the ingredients of products and conditions of manufacture.

Rheological properties

Rheological properties of dough were evaluated using Farinograph apparatus (Table 4). Water absorption, arrival time, dough-development time, and dough-weakening were increased, however dough-stability was decreased parallel with an increment of the WF-substitutional level using the MRS, SRB, and BB. This was joined to the high fiber content of the MRS, SRB, and BB compared to the WF. The fibers tend to bind water, and change the rheological characterization likely as the result represented of Kim et al. [21]. Resistance to extend, extensibility, and dough energy was decreased by different blended ratios of the MRS, SRB, and BB. The viscoelastic property of the WF-dough depends on gluten quality and quantity. Therefore, as gluten content increased the viscoelastic property was improved. The proportional number increased as the increment in percentage of the WF.

Table 3: Replacing of the WF with 10 % of the BB, MRS or SRB effect on snacks volatile compounds

Volatile compound	RI[a]	Control	BB	MRS	SRB
2-Methyl propanal	802	0.92[b]	0.78	0.45	1.73
2-Butanone	891	3.45	2.31	3.06	1.96
2-Methyl butanal	902	7.94	6.92	5.03	3.85
3-Methyl butanal	907	11.48	9.34	7.59	6.84
2-Ethylfuran	938	0.54	1.62	3.82	2.97
Pentanal	969	3.47	1.95	1.06	1.45
2,3-Butanedione	1058	26.84	23.47	15.03	19.38
Hexanal	1076	4.72	6.98	7.46	9.34
2-Methyl-1-propanol	1098	0.28	1.31	n.d	3.95
2-Butylfuran	1134	0.92	1.05	5.89	2.73
1-Butanol	1157	1.54	0.89	n.d	1.84
1-Penten-3-ol	1168	0.78	2.41	4.09	3.69
α–Terpinene	1172	1.06	0.67	1.72	4.82
2-Heptanone	1179	0.59	1.29	0.67	2.91
Heptanal	1184	2.51	1.53	3.05	3.05
3-Methyl-1-butanol	1213	0.34	0.89	3.46	3.24
2-Pentylfuran	1235	1.06	1.32	2.93	n.d
3-Methyl-3-buten-1-ol	1257	0.93	1.65	0.52	2.65
1-Pentanol	1265	1.02	0.78	1.68	1.83
Hexyl acetate	1279	4.76	2.97	0.04	n.d
α–Terpinolene	1283	0.07	3.05	2.71	1.59
3-Hydroxy-2-butanone	1289	5.32	3.72	2.93	n.d
Octanal	1295	1.93	0.04	1.26	4.29
1-Hydroxy-2-propanone	1302	0.67	1.83	0.74	2.71
2-Ethyl-1-butanol	1314	0.95	0.62	1.05	0.68
4-Methyl-1-pentanol	1326	1.42	1.19	6.03	2.04
2-Heptenal	1332	2.75	0.85	1.95	1.32
3-Methyl-2-buten-1-ol	1338	0.83	1.02	0.74	2.76
Ethyl heptanoate	1342	1.09	3.94	6.02	1.84
1-Hexanol	1385	0.68	5.91	3.98	0.95
Nonanal	1392	3.82	0.06	0.42	0.32
Ethyl octanoate	1431	0.78	1.57	0.76	0.61

The volatiles were determined in the snacks of 10% substitutions according the acceptability of consumers in the test panel assay and the overall acceptability results, [a]: RI retention indices; [b]:Values are expressed as relative area percentage, BB: barley bran, SRB: stabilized rice bran, MRS: modified rice bran, n.d: Not detected.

Table 4: The rheological parameters determined for the control-dough and substituted doughs of the MRS, SRB, and BB using the Farinograph and Extensograph

Samples	Farinograph parameters						Extensograph parameters			
	Water absorption (%)	Arrival time (min)	Dough development time (min)	Stability time (min)	Weakening (BU)	Mixing tolerance index (BU)	Extensibility (E) (mm)	Resistance to Extension (R) "BU"	Ratio (R/E)	Energy (Cm)2
WF (Control)	56	2.0	3.5	15.0	90	40	140	350	2.50	90
Snacks in which the WF replaced by the BB										
5%	58.0	2.5	4.0	13	95	45	120	320	2.67	80
10%	61.5	3.0	5.0	11	100	55	110	280	2.55	70
15%	63.5	3.5	5.5	10	110	65	90	250	2.78	65
20%	66.0	4.5	6.0	8.5	120	70	80	230	2.88	60
Snacks in which the WF replaced by the MRS										
5%	58.5	2.5	4.0	14.0	100	45	130	310	2.38	80
10%	61.5	3.0	5.0	12.0	120	55	120	280	2.33	70
15%	63.0	3.5	6.0	10.0	130	60	100	260	2.60	60
20%	65.0	4.0	6.5	8.5	140	70	85	240	2.82	55
Snacks in which the WF replaced by the SRB										
5%	59.0	3.0	4.5	13	100	50	125	320	2.56	85
10%	62.0	3.5	5.0	10	110	60	115	300	2.61	70
15%	64.0	4.5	5.5	8.0	130	65	100	280	2.80	65
20%	66.0	5.0	6.0	7.5	140	75	90	260	2.89	55

The values of each reading in the table was calculated as mean of three readings, The wheat flour substitutions were applied (at 5, 10, 15, and 20%), for each material (as mix of extracted powder and its extract content), WF: wheat flour, BB: barley bran, SRB: stabilized rice bran, MRS: modified rice bran.

Antimicrobial Activity of the MRS, SRB, and BB.

Antifungal and antibacterial activity of three byproducts (BB, MRS, and SRB) against pathogenic bacteria and toxigenic fungi were evaluated as shown in Table 5. Pathogenic bacteria were represented using six strains, which showed inhibition growth ability for the three types of byproducts.The recorded values of arbitrary units were ordered in ascending according to bacterial sensitivity for extracts as follows: *C. albicans* ATCCMYA-2876, *S. aureus* NCTC 10788, *S. senftenberg* ATCC 8400, *E. coli* BA 12296, *C. botulinum* ATCC 3584, and finally *B. subtilis* DB 100 host. While investigated toxigenic fungi strains recorded more extracts sensitivity (less arbitrary units). The inhibition effect of SRB, BB, and MRS manifested more antifungal characteristics than antibacterial ones. Therefore, this finding recommends applying SRB, BB, and MRS to achieve the effect of antifungal activity.

Table 5. Antimicrobial activity (AU/mL) calculated as arbitrary unit for the MRS, SRB and BB

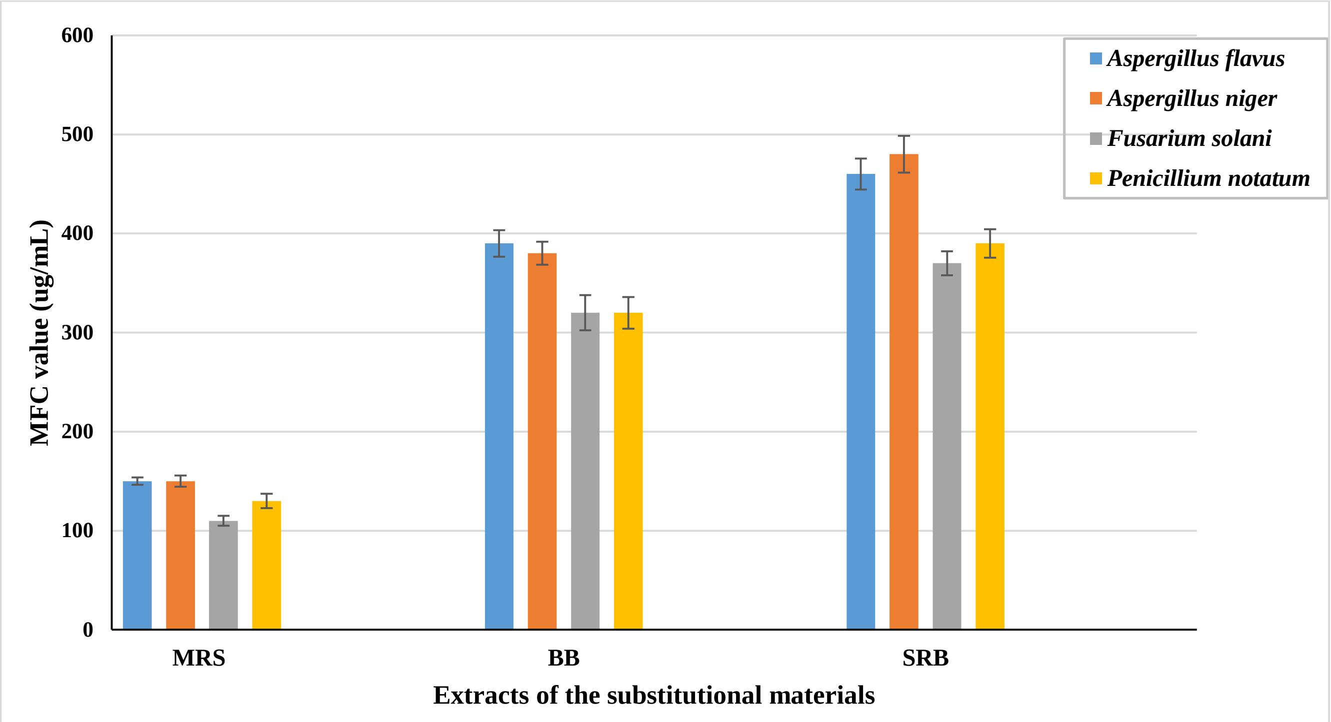
Indicator strains	MRS	SRB	BB
A. niger ITEM 3856	1850	4200	8400
A. parasiticus ITEM 11	2200	6300	12500
A. carbonarius ITEM5010	2600	3800	12800
A. flavus ITEM 698	2400	3600	14400
A. oryzae ITEMB5	1850	3200	10700
F. oxysporum ITEM 12591	2400	5600	10500
S. senftenberg ATCC 8400	4700	8400	19600
C. albicans ATCCMYA-2876	2800	4700	17500
*Cl. Botulinum*ATCC 3584	5800	10600	22000
B. subtilis DB 100 host	6200	7900	20400
S. aureus NCTC 10788	3200	5400	20800
E. coli BA 12296	5700	8000	22300

Extracts were applied at concentrations of 10% (v/v), BB: barley bran extract, SRB: stabilized rice bran extract, MRS: modified rice bran extract.

Antifungal activity

To confirm the antifungal characteristics of the SRB, BB, and MRS, it was applied in YES media contains four local isolated strains of mycotoxigenic fungi (Fig. 1A). The autoclaved materials of MRS, SRB, and BB were inserted into the liquid media at 20% (w/v). Inoculated flasks incubated for 5 days at 28°C. Results indicated that; the higher antifungal potency was recorded for MRS compared to SRB and BB. The antifungal effect was represented as decreases in the mycelia weight of fungal growth against the control. The SRB has the lowest antifungal potency in the case of applied toxigenic fungi. The obtained results emphasize the antifungal properties of MRS, SRB, and BB against toxigenic fungi that had an ability for producing mycotoxins.

A



B

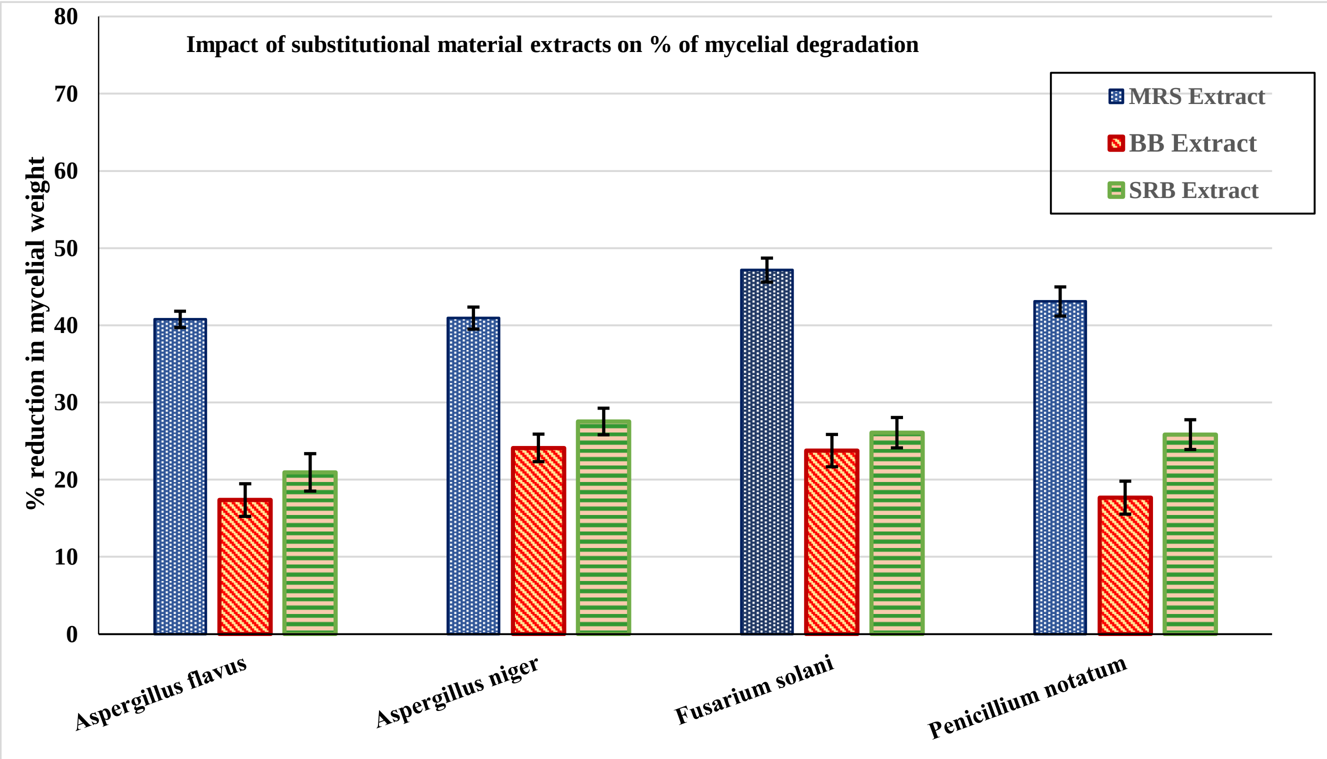


Figure 1. Evaluation of the substitutional extracts for their antifungal properties against the toxigenic fungi
(A) Minimal fungicidal concentrations (MFC) of SRB, BB, and MRS on toxigenic fungal growth (measured as µg extract/mL of growth medium), where the values for the standard Mycostatin (+ control) was recorded at 0.16µg/mL media).
(B) Inhibitory effect of substitution material extracts on fungal mycelial weight (% of reduction in weight).
* SRB: Stabilized rice bran
* MRS: modified rice straw
* BB :barley bran

The MFC indicated of the minimal volume of testing extracts, which were able to inhibit the fungal growth of toxigenic fungi. The MFC values for SRB, BB, and MRS were estimated to explore and ensure the best component for antifungal properties (Fig. 1B). In agreement with the antifungal results represented in previous experiments in this study, the MRS showed again the best MFC impact. The MFC value of MRS extract varied between 110 and 150 mg/Kg compared to the standard fungicide drug of Nystatin. The lowest antifungal activity measured as MFC was recorded at 480 mg/Kg for the SRB.

Inhibition of aflatoxin secretion

The reduction of the AFs secretion and the inhibition impact of the SRB, BB, and MRS were evaluated against a high AFs-producing strain (*A. Paraciticus* ITEM 11). The reduction-impacts for the MRS, SRB, and BB were estimated individually, and at various concentrations (5%, 10%, 15%, and 20%). The data represented in Fig. 2 illustrated the ability to insert the byproducts to reduce AFs-secretion in the inoculated media. The data recorded in Fig. 2-a showed the highest AFs excretion reduction by the presence of MRS in growth media. By an increase of MRS concentration, presence in the media, the AFs reduction increased. The increases of the BB concentration in liquid growth media recorded none significant differences of AFs reduction excreted by the fungi (Fig. 2-b). Concerning the SRB application in growth media that was inoculated by the AFs producer strain *A. parasiticus* ITEM 11, a reduction effect was raised by concentration elevating of SRB in media (Fig. 2-c).

Substitutional snacks resistance to fungal contamination

The evaluated results of packed substitutional snacks reflect the efficiency of the MRS-snacks to delay the fungal colony appearance. For the accelerated contaminant samples of snacks (Fig. 3), it was taken up to 5 days until the fungi-colonies were formed on the snack service. The colony-formation for the BB-snacks and SRB-snacks was manifested after a 3 and 2 days, respectively. The most resistant snack was recoded for the propionate-snacks (7 days). Otherwise, negative control snacks that were left for the natural contaminate, in sealed-pack, the occurrence of fungal growth was recorded after 32 days of storage at room temperature. The obtained results for proximate analysis of substitutional materials and wheat flour reflect the distinguished components, mainly for the MRS. It was recorded by high contents of fiber fractions, total phenolic, antioxidants activity , and the (a*) value that represents red attributes of the MRS. Besides, the evaluation of MRS-antimicrobial and antifungal characteristics were manifested by the best potency. This was explained due to its wealthy volatile-compound profile, with a special content of compound possessed antifungal and antimicrobial properties.

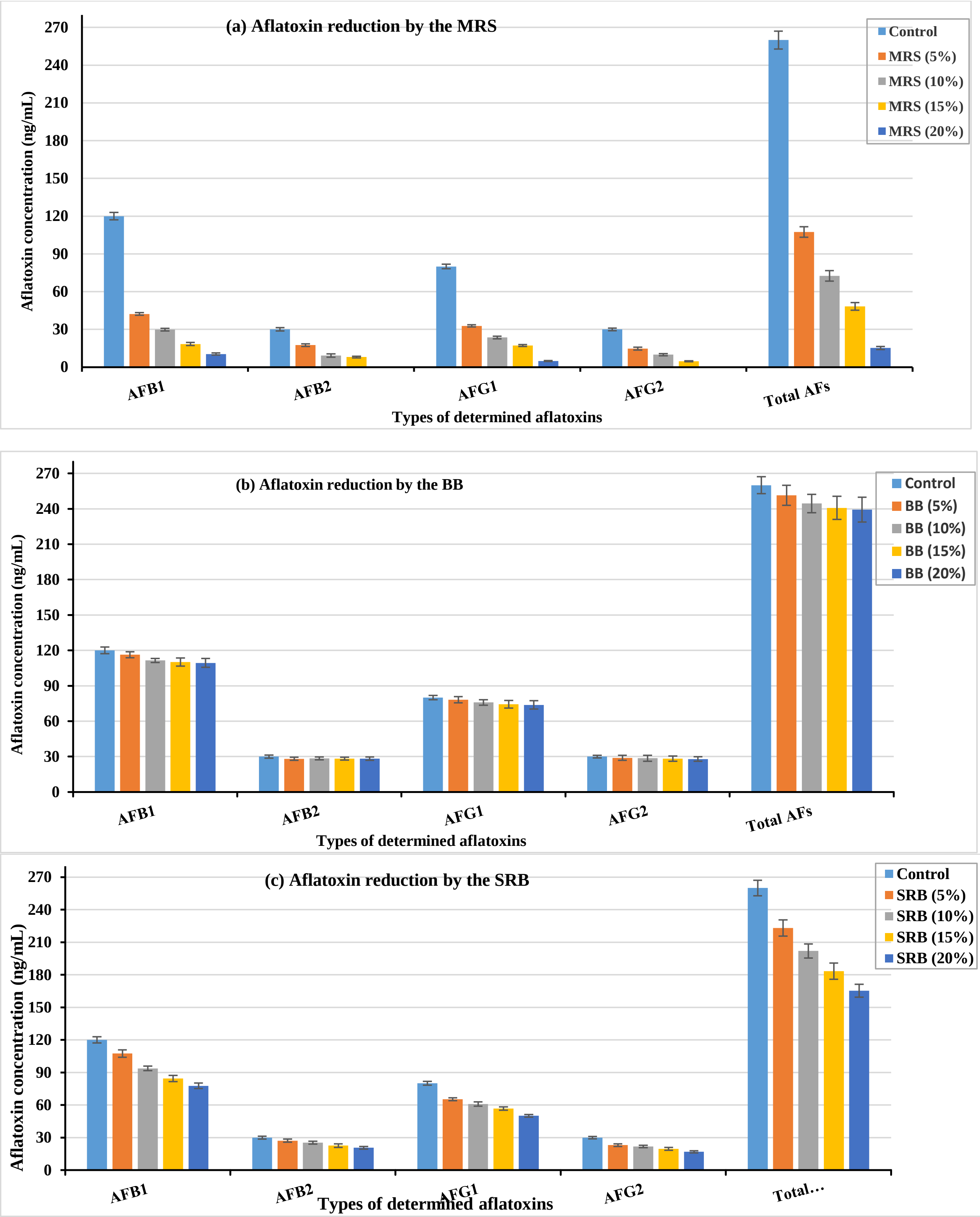


Figure 2. Aflatoxin reduction by (a)MRS, (b) BB, and (c) SRB extracts at various concentrations (5% to 20%).

* SRB: Stabilized rice bran
* MRS: modified rice straw
* BB :barley bran

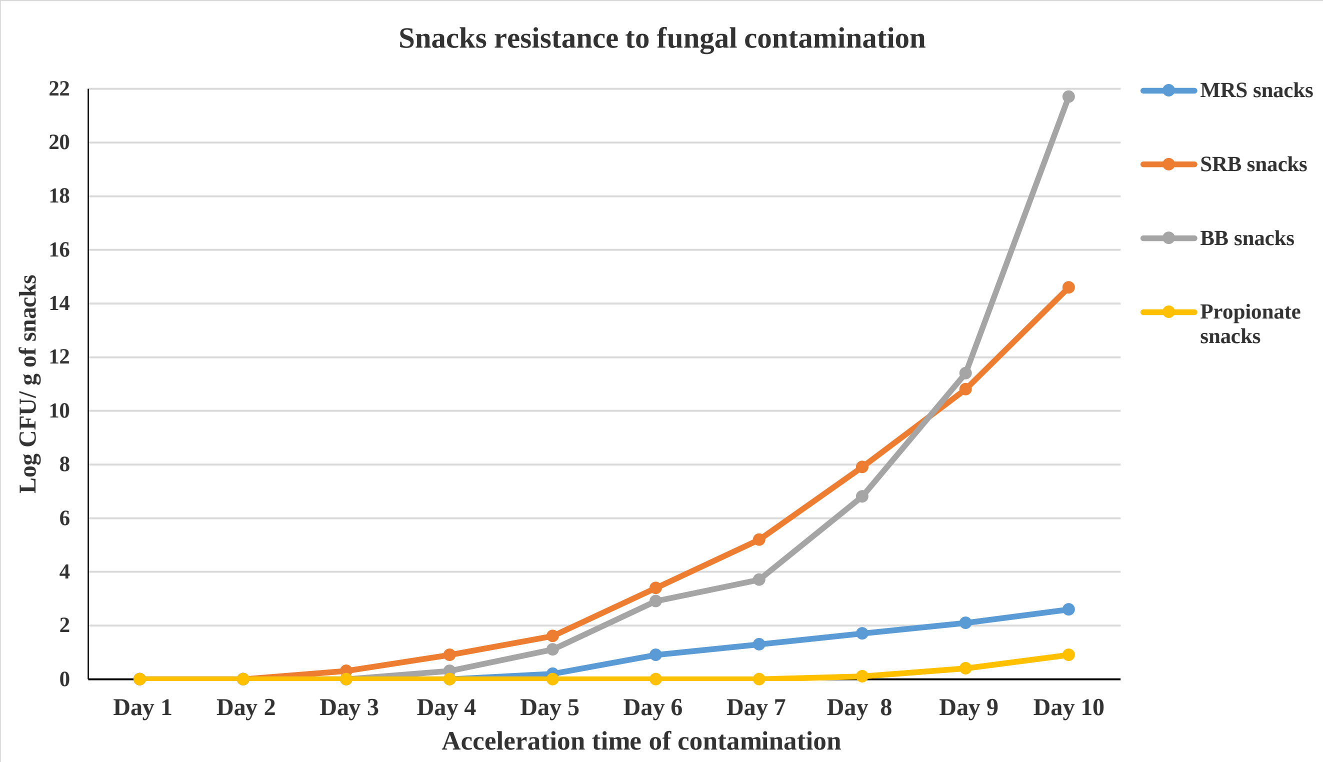


Figure 3: The effect of MRS, SRB, and BB on extended the shelf life of substitutional snacks against toxigenic fungal contamination compared to preservative snacks by propionates.

For the explanation of obtained volatiles-result, it could demonstrate because of the thermal process of substituted MRS-snacks. The elevation recorded for the components like 2-Ethylfuran, 2-butylfuran, 2-3 Butane-Dione, α–Terpinene, and α–Terpinolene, Hexanol, and Hexanal. The volatiles were manifested an antifungal and antimicrobial properties, particularly against toxigenic fungi. The increment in various volatiles concentrations, as represented in Table 3**,** explain the aid-effects against the fungal growth, as well as, their AFs-secretion reduction. The presence of volatile compounds in the SRB and BB-snacks could play the same role for fungal inhibition and aflatoxin formation reduction, but in a less impact related to their volatiles or other contents. The AFs affected the food and biochemical system through its oxidative stress impact [22]. The increased values of the antifungal-volatiles may help in suppressing the oxidative stress impact.

The impact was related to the presence of bioactive compounds (phenolic and antioxidant phenolics), which were played a vital function and assisted in suppressing the fungal contamination of substituted snacks. The oxidative stress and free radicals are considered a factor that affecting the fungal-metabolism of toxin secretion during their growth. The phenolic and phytochemical bioactives existence were influenced the fungi stress, and could redirect the fungi to stop their secretion of secondary metabolites. Again, phenolic content in the product may exercise oxidative stress against aflatoxin hazard [23].

The existence of volatiles were possessed an aid-impact by their fungal inhibition effect. The reduction of fungal growth mainly was joined to aflatoxin-secretion amount. This theory could demonstrate how the substitutional materials of SRB, MRS, and BB participate in the reduction of AFs-formation. Fiber-fractions also were known by efficiency in mycotoxin degradation, lignin and cellulose were the identified fraction possess a limitation influence on the AFs-contamination.

Regarding the authors' the increment in the amount of these components, the increase in fungal inhibition recorded and the more limited of aflatoxin formation. In this regard, the substitutional of the WF by the MRS for the industrial scale of the snack production could provide a more-safety product, besides the enhancement of nutritional and flavoring characteristics. This was ensured by the accelerated-contaminated experimental, in which the resistance of MRS-snacks to fungal contamination was recorded close to the resistance of chemical-preservative snacks with propionates.

Conclusion

The suitable percent for by-products substitution in the present study is 10 percent. This percent is contains large amount of phenolic compounds with antioxidant activity and also produce volatile compounds. Both of them besides fiber are effective as antifungal. The produced snacks by this addition is acceptable. However, the MRS was appeared contains phenolic, antioxidants, volatile components with antifungal properties, and fiber fractions. It was manifested ability to resist the fungal contamination in the acceleration experiment.

Acknowledgement: This work was totally supported through the research project No. 11040 113 funded by the National Research Center, Cairo, Egypt.

Conflict of interest

The authors have no conflict of interest.

References

1. Hussein, A.M.; Ibrahim, G.E.. Effects of various brans on quality and volatile compounds of bread. Foods and Raw Materials, 7, 35 - 41(2019).
2. Sharif, M., Butt, M., Anjum, F.; Khan, S. Rice bran: A novel functional ingredient. Crit. Rev. Food Sci. Nutr., 54, 807-816(2014).
3. Gul, K., Yousuf, B., Singh, A., Singh, P.; Wani, A.A. Rice bran: Nutritional values and its emerging potential for development of functional food—A review. Bioactive Carbohydrates and Dietary Fibre,6, 24-30(2015).
4. Martínez-Monzo, J., García-Segovia, P.; Albors-Garrigos, J. Trends and innovations in bread, bakery, and pastry. J. Culinary Science & Technology, 11, 56-65(2013).
5. Badr, A.N.; Naeem, M.A.. Protective efficacy using Cape- golden berry against pre-carcinogenic aflatoxins induced in rats. Toxicology Reports, 6, 607-615(2019).
6. Shehata, M.G., Badr, A.N., El Sohaimy, S.A., Asker, D.; Awad, T.S. Characterization of antifungal metabolites produced by novel lactic acid bacterium and their potential application as food biopreservatives. Ann. Agric. Sci, 64, 71-78(2019).
7. Mohamed, S., El-Desouky, T., Hussein, A., Mohamed, S.; Naguib, K. Modified Rice Straw as Adsorbent Material to Remove Aflatoxin B_1 from Aqueous Media and as a Fiber Source in Fino Bread. Journal of toxicology.2016, 1-10(2016).
8. AACC. Approved methods of the AACC(10th ed.). In St. Paul (Ed.), American Association of Cereal Chemists, Vol. 1 ; Methods 10-50.05, 32-05.01, 32-07.01, 32-20.01, 33-50.02, 54-10.01, 54-10.02. Pilot Knob Road; St. Paul, MN 55121 USA. (2000).
9. Singleton, V.L. ,Orthofer, R., Lamuela-Raventos, R.M..Analysisof total phenols and other oxidation substrates andantioxidants by means of Folin–Ciocalteu reagent, Methods Enzymol. 299: 152–178. (1999).
10. Wójtowicz, A., Zalewska-Korona, M., Jabłońska-Ryś, E., Skalicka-Woźniak, K.; Oniszczuk, A. Chemical Characteristics and Physical Properties of Functional Snacks Enriched with Powdered Tomato. Polish J. Food Nut. Sci. 68(3), 251-261(2018).
11. Adams, R. Identification of essential oil components by gas chromatography/mass spectrometry, Allured Publishing Carol Steam Ilionois, USA. (2007).
12. Dellavalle, P.D., Cabrera, A., Alem, D., Larranaga, P., Ferreira, F.; Dalla Rizza, M. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chilean J Agri. Res., 71, 231-239(2011)..
13. Aoudia, N., Callu, P., Grosjean, F.; Larondelle, Y. Effectiveness of mycotoxin sequestration activity of micronized wheat fibres on distribution of ochratoxin A in plasma, liver and kidney of piglets fed a naturally contaminated diet. Food Chem. Toxicol., 47, 1485-1489(2009).
14. Mc-Clave, J.; Benson, P. Statistics for Business and Economics. Dellen Publishing, San Francisco, Calif, USA; Max Well Macmillan, New York, NY, USA. (1991).
15. Jalili, M. A review on aflatoxins reduction in food. Iranian J. Health, Safety & Environ., 3, 445-459(2016).
16. Fountain, J., Bajaj, P., Nayak, S., Yang, L., Pandey, M., Kumar, V.; Kemerait, R. Responses of Aspergillus flavus to oxidative stress are related to fungal development regulator, antioxidant enzyme, and secondary metabolite biosynthetic gene expression. Frontiers in microbiology, 7, (2048)1-16(2016).
17. Carroll, L. Functional properties and applications of stabilized rice bran in bakery products. Food Technology, 44, 74-76(1990).
18. Nawwar, G., Maher, A., Al-Hashash, Swellam, R.; Ahmed, H. Design and facile synthesis of new

bioactive C-glycosidic semi natural compounds. Egypt J. Chem., 63, 1:185 – 195(2020).

19. Oruna-Concha, M., Bakker, J.; Ames, J. Comparison of the volatile components of eight cultivars of potato after microwave baking. LWT-Food Science and Technology, 35, 80-86(2002).

20. Birch, A.N., Petersen, M.A.; Hansen, A.S. The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. LWT-Food Science and Technology, 50, 480-488(2013).

21. Kim, Y.S., Ha, T.Y., Lee, S.H.; Lee, H.Y. Effect of rice bran dietary fiber on flour rheology and quality of wet noodles. Korean J. Food Sci. Technol., 20, 90–95(1997).

22. El-Nekeety, A.A., Mohamed, S.R., Hathout, A.S., Hassan, N.S., Aly, S.E.; Abdel-Wahhab, M.A. Antioxidant properties of Thymus vulgaris oil against aflatoxin-induce oxidative stress in male rats. Toxicon, 57, 984-991(2011)..

23. Hathout, A.S., Mohamed, S.R., El-Nekeety, A.A., Hassan, N.S., Aly, S.E.; Abdel-Wahhab, M.A. Ability of *Lactobacillus casei* and *Lactobacillus reuteri* to protect against oxidative stress in rats fed aflatoxins-contaminated diet. Toxicon, 58, 179-186(2011)..

دراسة مقارنة لتقييم تأثير إضافة بعض النواتج الثانوية على المقرمشات من حيث مركبات النكهة الطيارة وتقليل إنتاج الأفلاتوكسينات

أحمد سعيد حسين [1] – جميل السيد إبراهيم [2] – أحمد نوح بدر [3]

[1] : قسم الصناعات الغذائية – المركز القومى للبحوث - الجيزة - مصر

[2] : قسم كيمياء مكسبات الطعم والرائحة - المركز القومى للبحوث - الجيزة - مصر

[3] : قسم سموم وملوثات الغذاء - المركز القومى للبحوث- الجيزة – مصر

تعتبر المقرمشات من الوجبات التقليدية أوتستهلك بين الوجبات عن طريق الأطفال أوالبالغين ولذا فمن الأهمية تدعيم هذه المنتجات لزيادة خصائص سلامتها وإطالة فترة الصلاحية بدون التأثير على قابيتها. في هذه الدراسة تم تدعيم المقرمشات عن طريق قش الأرز المعدل ، ردة الأرز المثبتة وكذلك ردة الشعير لإستبدال دقيق القمح في صناعة المقرمشات. تم تقييم تأثير السلامة الميكروبيولوجية للمواد المضافة وتقليل إفراز الأفلاتوكسينات وكانت كفاءة تثبيط الفطريات المنتجة للتوكسينات هي كالتالى قش الأرز المعدل ثم ردة الأرز المثبته وأخيرا ردة الشعير. سجلت نتائج التقييم الحسي أقل تقييم في العينات المعاملة بالتركيزات العالية من ردة الأرز المثبته وقش الأرز المعدل. كانت أفضل نتائج التقييم الحسي في العينات المعاملة بتركيز 10 % كبديل لدقيق القمح ولذلك تم تقدير مركبات النكهة الطيارة فيها بجهاز التحليل الغازى الكروماتوجرافى – طيف الكتلة . أظهرت النتائج أن مركبات النكهة السائدة كانت هي 2,3-butanedione, 3-methyl butanal, and 2-methyl butanal بتركيزات 26.84% ، 11.48% ، 7.94% على التوالي. يعزى إنخفاض إفراز الأفلاتوكسينات إلى مركبات النكهة الطيارة.

الكلمات الدالة: الردة – المقرمشات – النكهة - الأفلاتوكسينات