FJARD VOL. 36, NO. 3. PP. 313-323 (2022)

Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790
On Line ISSN:2805-2528

Effect of baking process on the benzoquinones secreted by the confused flour beetles, *Tribolium confusum* (Du Val), in biscuits made from different types of infested flour

Salwa S. Pasha[1*], M. I. M. Ibrahim[2], Abdelmonam M.A. Abu El-Hassan[3], Samah A. Abd-Eltawab[3]

[1]Plant Protection Dept., Faculty of Agriculture, Fayoum University, Egypt
[2]Food Toxicology and Contaminants Dept., National Research Center, Dokki, Giza, Egypt
[3]Food Science and Technology Dept., Faculty of Agriculture, Fayoum University, Egypt

ABSTRACT:

The experiment was conducted to estimate the levels of benzoquinones in biscuits that were baked at 180 ± 5°C for 20 minutes with five different types of flour: wheat flour, barley, white maize, sorghum and oats, infested with the confused flour beetles, *Tribolium confusum* (Du Val) and stored for three months.

The results showed the presence of high levels of benzoquinones in all types of biscuits. Barley biscuits recorded the highest percentage at 11.10 mg/g, followed by wheat 6.27 mg/g, white maize 5.10 mg/g, sorghum 2.72mg/g and oats 0.95 mg/g, respectively. Also the data showed a positive correlation between the total number of insects in the infested flour before baking and the level of benzoquinones after baking. The cracking of benzoquinone by the heat of the oven during the baking of biscuits resulted in many harmful organic compounds, which were identified by analysing it on GC/MS. All of the major compounds were present in large percentages in all of the biscuit samples, namely: 1,4,4-trimethylcyclohexa-2-en-1-ol, which represented the highest percentage of the separated compounds, with a peak area percentage ranging from 41.95-50.92%; 2-Octenal, 2-butyl, which represented a percentage of 21.85-23.64%; 7-Octen-4-one, 2,6-dimethyl, which represented a percentage of 12.55-17.54%; valeric acid, 4-tridecyl ester, represented by 6.25–9.14%; phenol area percentage was 3.53–7.48% and area percentage of Carbamic acid phenyl ester was 0.55–3.03%. In addition to 6 other minor compounds. We concluded that made from infested flour are extremely dangerous due to the presence of benzoquinone and many other organic compounds produced by heat cracking. It also showed the sensory evaluation of the biscuits made from infected flour revealed an undesirable taste, odor and darkening in color. Commercial fraud exposes us to the same risk by combining infested flour or bran with non-infested flour.

Keywords: Benzoquinone, Thermal demolition, *Tribolium confusum*, Infested Biscuits, HPLC, GC-Ms.

* Corresponding author: ssb01@fayoum.edu.eg
Received: 6/7/ 2022
Accepted: 3/8/ 2022

1. INTRODUCTION:

Flour beetles, including the confused flour beetle, *Tribolium confusum* (Du Val), attack wheat flour, cereal products, and food dried in mills or stores. The abdominal and thoracic odoriferous glands of both sexes of adult flour beetles secrete a foul-smelling substance known as quinones (**Roth and Howland, 1941**).

Tribolium confusum (Du Val) produces two compounds: methyl-1,4-benzoquinone (MBQ) and ethyl-1,4-benzoquinone (EBQ). These compounds play a role as external defences to kill microbes and predators **(Ruther et al., 2001; Prendeville and Stevens, 2002)**. The infested flour becomes distasteful smelling and pink in colour due to contamination by this **secretion (Engelhardt et al., 1965).**

Previous studies indicated that the quinones secreted by flour beetles have a carcinogenic effect on several organs and lower antioxidant enzymes in experimental animals and humans **(El-Mofty et al., 1988; El-Mofty et al., 1992; Elhassanee and Abd El-Moa, 2003)**.

It also contributes to the dissemination of microorganisms in food, particularly mycotoxigenic fungus **(Weston and Rattlingourd, 2000)**. Food contamination with insect bodies and their waste leads to allergic reactions or poisoning in some cases **(Freeman, 1976)**.

The present work aims to measure the residue BQs in biscuits made with infested flour, which are secreted by *Tribolium confusum* in flour infested as well as the effect of oven temperature on these secretions (BQs) after baking biscuits.

2.MATERIALS AND METHODS:

Insect source

The adults of *T. confusum* used in this experiment were obtained from a stock culture reared in stored whole wheat flour at the laboratory conditions in the Plant Protection Dept., Faculty of Agriculture, Fayoum University, Egypt.

Flour sources

Fifth different flours were tested, including whole wheat flour (*Triticum aestivum* L.) with an extraction rate of 82%. It is manufactured in the mills of the Arab Republic of Egypt. The whole grains of white maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and sorghum (*Sorghum bicolor*) belong to the grass family Poaceae. They were obtained from local market in Fayoum governorate, Egypt, and crushed by an electric grinder.

Experiment Procedure

Ten pairs mixed sex of confused beetle adults, *T. confusum*, were released into small glass jars (5 cm in diameter and 12 cm in length) with 40 gm of each type of flour in three replicates, as well as three replicates of glass jars free from insects as the control. The jars were covered with muslin cloth and incubated in the laboratory incubator for three months at (28 ± 2 ° C).

After the incubation period, the flours of each replicate were sieved with a wire sieve to separate the different stages of insects and counted. The three exact replicates of each type of flour were collected together and weighed.

Biscuits preparing

After The three exact replicates of each type of flour were collected together. The biscuits were prepared in the laboratory using 100 gm of each infested flours , 30 gm of honey bee, 30 ml of sunflower oil and baking powder. The ingredients were kneaded well, formed, and each type was placed in a separate dish, finally baked in an oven at 180 °C for 20 min.

After baking time the biscuits left to cool to room temperature, then weighed and crushed separately using a manual mortar and pestle..

Extraction and determination of benzoquinones.

Benzoquinone (BQ) were extracted and determined according to **(Tomoskozi-Farkas and Daood, 2004)**. Benzoquinones are extracted from the equivalent to 2 g of flour from biscuit weight. After grinding the sample, 50 ml doubly distilled water were added and shacked well, then extracted three times by 25 ml chloroform. The chloroform layers were pooled, then washed twice by distilled water and dried over anhydrous sodium sulphate (Na2SO4), and filtered by using Whatman filter paper. The filtrate was evaporated to dryness by rotary evaporation at 40 °C. The dry material was finally redissolved in 10 ml of methanol.

The HPLC was used for the determination of benzoquinones in baked biscuits according to **(Tomoskozi-Farkas and Daood, 2004).**

Analysis of gas chromatography-mass spectrometry (GC-MS) was used to estimate any change in the compounds secreted by the beetles (benzoquinones) in infested baked biscuits as a result of exposure to heat.

At the Central Laboratories Network, National Research Centre in Giza, Egypt, the GC-MS system (Agilent Technologies) was outfitted with a gas chromatograph (7890B) and a mass spectrometer detector (5977A). The HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 m film thickness) was installed in the GC. Hydrogen was used as the carrier gas in the analyses, with a flow rate of 2.0 ml/min at a splitless, injection volume of 1 l, and the following temperature programme: 60°C for 1 minute; rising at 20°C/min to 100°C and holding for 0 minutes; rising at 30°C/min to 250°C and holding for 0 minutes. The injector and detector were kept at 300 °C. Electron ionisation (EI) at 70 eV was used to obtain mass spectra with a spectral range of m/z 25-700 and a solvent delay of 2 minutes. The mass temperature was 230°C and the quad temperature was 150°C. By comparing the spectrum fragmentation pattern to those stored in the Wiley and NIST Mass Spectral Library data, the different constituents were identified.

These experiments were conducted at the Food Toxicology and Contaminants Dept., National Research Center, Dokki, Giza, Egypt.

Sensory evaluation of biscuit

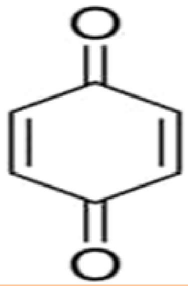
Ten panel tastes from the Food Science and Technology Department at the Agriculture Faculty at Fayoum University evaluated the biscuit samples. Larmond gave the biscuits a score of 10 for overall acceptability, color, taste, texture, and odour. A numerical hedonic scale ranging from 1 to 10 (1 being very bad and 10 being excellent) was used for sensory evaluation **(Larmond, 1977)**.

Data analysed

A Pearson's simple correlation test was conducted between the total number of *T. confusum* adults in infested flour and the levels of benzoquinones in the baked biscuit made from infested flour by same insect. Sensory characteristics were statistically analysed using one-way analysis of variance with "Duncan test" at the 0.05 probability level by using the IBM SPSS 21.0 Statistical Package for Social Sciences.

3. RESULTS AND DISCUSSIONS:

Qualitative and quantitative analysis of benzoquinones by HPLC



p-Benzoquinone

The HPLC analysis of the samples of biscuits made with flour infested by *T. confusum* adults showed the presence of a peak from 1, 4 benzoquinone (p - benzoquinone). Several Coleopteran beetles, particularly *Tribolium* spp., have defence glands that secrete quinones **(Howard, 1987; Li et al., 2013)**. *Tribolium's* volatile defence secretions have already been well described **(Villaverde et al., 2007)**. The 3 quinones isolated from the adult beetles, *Tribolium castaneum*, 80-90% were 2-ethyl-l, 4,-benzoquinone 10-20% were 2-methyl-l, 4-benzoquinone, and a trace of 2-methoxy-l,4-benzoquinone **(Loconti and Roth, 1953)**. *Tribolium confusum* produces two compounds methyl-1, 4-benzoquinone (MBQ) and ethyl- 1, 4-benzoquinone (EBQ) **(Yezerski et al., 2004)**. The percentage of benzoquinone secreted by *Tribolium confusum* was 73%, and the ratios of 2-methyl- to-2-ethyl-1, 4-benzoquinone were 1: 2.7 **(Markarian et al., 1978)**.

Benzoquinone levels were also determined as shown in Table 1 & Fig. 1. Barley biscuits recorded the highest percentage of benzoquinones at 11.10 mg/g, followed by wheat, white maize, and sorghum biscuits as follows: 6.27, 5.10, and 2.72 mg/g, respectively, while the oats biscuits recorded the lowest level of BQs at 0.95 mg/g.

It is also noted from the data contained in (Table 1) that the concentration of benzoquinones is related to the number of insects. Where a significant positive correlation was observed between them (r =0.99), the level of benzoquinone increased with the increase in the number of insects.

Where he recorded the biscuits made from barley flour infested with the total of 646 insects, the highest percentage of benzoquinone was 11.10 mg (BQs)/g made biscuits. The percentage decreased successively with a decrease in the number of insects, recording the lowest percentage of the benzoquinone concentration of 0.95 mg/g in oats biscuits at the lowest level of infestation by 132 insects. Our findings are consistent with those of **Senthilkumar et al. (2012)** and **El-Desouky et al. (2018)**, who discovered that the rate of benzoquinone secretion increased with increasing insect densities of *T. castaneum.*

Table. 1. Levels of 1, 4 p- benzoquinone (BQ) in biscuits backed from different types of flour infested by *Tribolium confusum* after three months of storage.

Type of biscuits	Concentration of BQs ($C_6H_4O_2$) mg/g	Adults cumulative number
Control	ND	ND
Barley biscuit	11.10	646
Wheat biscuit	6.27	425
White maize biscuit	5.10	288
Sorghum biscuit	2.72	221
Oats biscuit	0.95	132
Pearson correlation "r"	0.99**	

ND: not detect

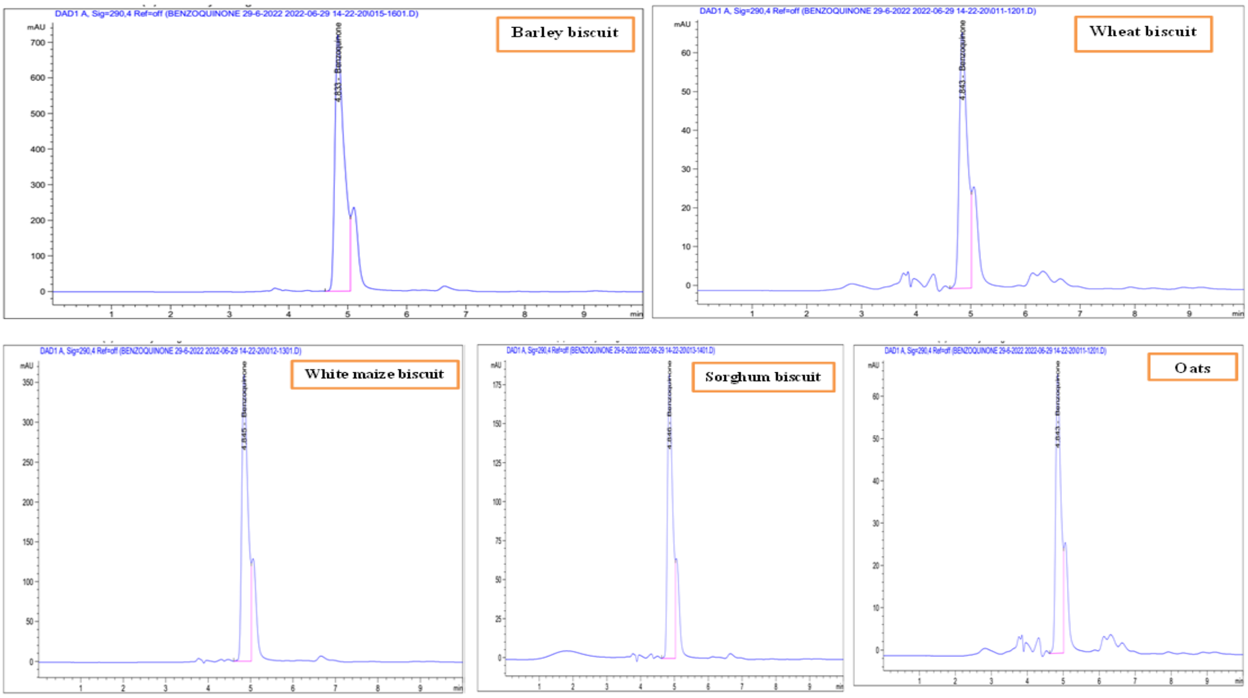


Fig. 1. Peaks of 1, 4 para benzoquinone (BQ) in the baked biscuits which identified and determined by HPLC

Chemical Analysis for fractions of benzoquinones by GC-MS

The cracking of benzoquinone by oven heat during baking of biscuits resulted in many harmful organic compounds, which were identified by their analysis on GC/MS.

Six major compounds were found in all types of tested biscuits. It is shown in Table 2 & Figure 2, where 1,4,4-trimethylcyclohexa-2-en-1-ol recorded the highest concentration in the derived compounds with a percentage ranging between 41.95% for wheat biscuits and 50.92% for sorghum biscuits out of the total percentage of recorded compounds. Its danger is that the LDLO (The lethal dose low) was 1 mL/kg on rabbits, which cause convulsions and death **(NTIS)**.

Followed by the compound 2-Octenal, 2-butyl-with a percentage ranging from 21.85% for sorghum biscuits to 23.64% for wheat biscuits, and previous studies showed that the toxicity of this compound on MTT assay of human neuroblastoma SK-N-SH cells showed that the IC_{50} value of 2-Butyl-2-octenal was 37.2 µM **(Yamada, et al., 2015).**

The chemical compound 7-Octen-4-one, 2,6-dimethyl, with a percentage ranging from 12.55% for sorghum biscuits to 17.54% for white maize biscuits. **Zhou, et al., (2022)** found that the LC50 (Lethal Dose) of 2, 6-dimethyl-7-Octen-4-one, on some Chinese fish juvenile *Gobiocypris rarus* was 48 (mg/L), this is considered moderately toxic.

While a peak area percentage of Valeric acid, 4-tridecyl ester was 6.25% for oat biscuits to 9.14% for white maize biscuits. Phenol ranked fifth in the list of main compounds, recording the highest percentage in wheat biscuits by 7.48% and the lowest percentage in sorghum biscuits by 3.53%. Phenols are dangerous ecotoxins that are mutagenic and carcinogenic to people and other living things, as well as hematotoxic and hepatotoxic **(Michałowicz, and Duda, 2007)**. The compound carbamic acid, phenyl ester occupied the last rank, with the highest percentage in oat biscuits by 3.03% and the lowest percentage in wheat biscuits by 0.35%.

Table 2. Major chemical compounds in infested biscuits were separated by GC-MS

Chemical compounds	1,4,4-trimethylcyclohexa-2-en-1-ol		2-Octenal, 2-butyl		7-Octen-4-one, 2,6-dimethyl		Valeric acid, 4-tridecyl ester		Phenol		Carbamic acid, phenyl ester	
Formula	$C_9H_{16}O$		$C_{12}H_{22}O$		$C_{10}H_{18}O$		$C_{18}H_{36}O_2$				$C_7H_7NO_2$	
Conc / **Type of biscuits**	**RT**	**Area Sum %**	**RT**	**Area Sum %**	**RT**	**RT**	**RT**	**Area Sum %**		**Area Sum %**	**RT**	**Area Sum %**
Barley biscuit	4.22	48.12	4.053	22.99	3.924	14.83	3.848	9.03	2.558	C_6H_6O	2.77	0.44
Wheat biscuit	4.243	41.95	4.083	23.64	3.954	16.42	3.886	8.92	2.649	RT	2.892	0.35
White maize biscuit	4.22	42.74	4.053	22.07	3.916	17.54	3.848	9.14	2.558	4.21	2.77	0.94
Sorghum biscuit	4.219	50.92	4.045	21.85	3.916	12.55	3.848	6.26	2.565	3.53	2.762	0.55
Oats biscuit	4.212	44.6	4.045	22.51	3.916	15.47	3.848	6.25	2.573	6.83	2.763	3.03

RT: retention time

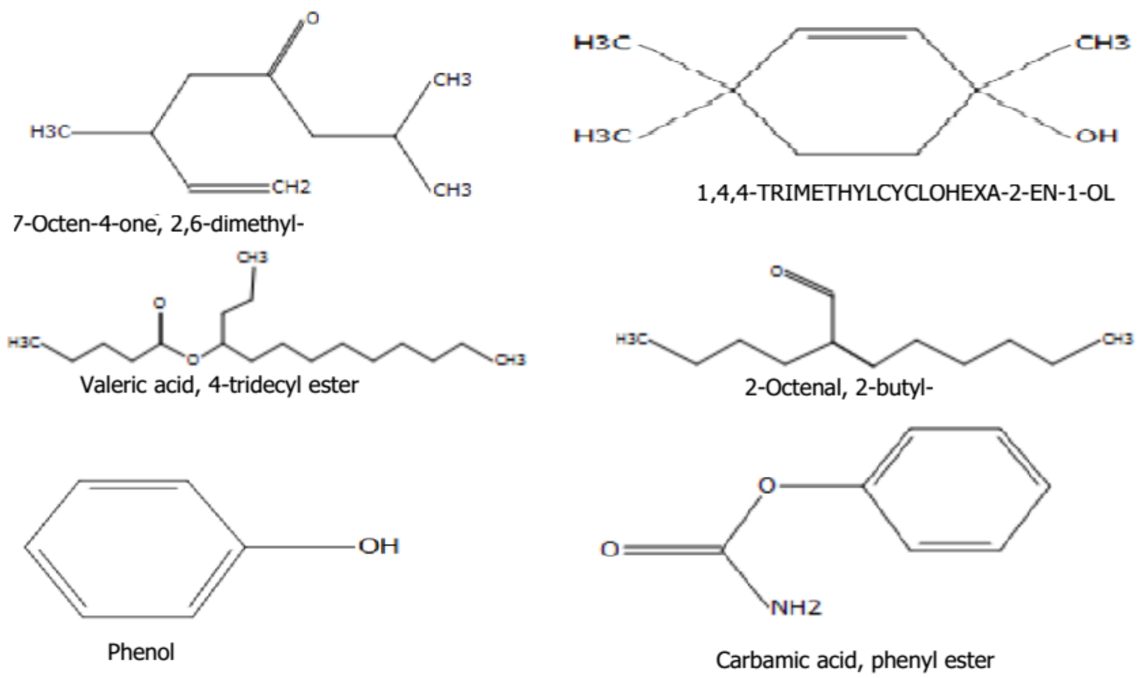


Fig. 2. Chemical structures of major compounds in infested biscuits which were separated by GC-MS

The data presented in Table 3 and Figure 3 show the types and percentages of the minor components in infested biscuits were derived from benzoquinones as a result of heat exposure.

1,1-Heptanediol diacetate, which was found in small amounts that did not exceed 0.5 percent in all types of biscuits, except barley biscuits, where this compound was not found., Isosorbide dinitrate was recorded in a very small percentage ranging between 0.11-0.36% in all types of biscuits except that it was not present in barley and oat biscuits. Benzeneethanol, α-methyl-3-(1-methylethyl) appeared only in wheat and white maize biscuit samples with a percentage of 0.33 & 0.13%, respectively. 2H-Pyran, 2-(2,5-hexadiynyloxy) tetrahydro was recorded only in oat and white maize biscuits with a percentage of 1.18 & 0.3 %, respectively. Only 0.55 and 0.66% of octanal and 2.09 and 2.32% of glycerin were detected in white maize and sorghum biscuit samples, respectively.

Flour beetles infesting a wide range of foodstuffs: rice, ground flour, raisins, nuts, fodder, and ordinary food, which is accompanied by their chemical secretions from the beginning of the invasion, and thus the danger of the spread of these substances between humans and animals increases.

Previous studies have shown that the affected flour is not suitable for human consumption and its health hazard. Because 1,4-benzoquinones are typical infesters of many human foods, the high percentages of 1,4-benzoquinones in *Tribolium confusum* secretions are problematic for food safety.

According to **Lee et al., (2013)**, 1, 4-benzoquinone is extremely toxic to humans and has been detected in rice infested with *Sitophilus oryzae* and *Tribolium castaneum*. Biscuits made from infested flour with *Tribolium castaneum* had the same carcinogenic potential as the flour infested. Moreover, oven temperature had no effect on the carcinogenicity **(El-Mofty et al., 1989)**.

Table 3. Minor chemical compounds in infested biscuits were separated by GC-MS

Chemical compounds	**1,1-Heptanediol, diacetate**		**Isosorbide Dinitrate**		**Benzeneethanol, α-methyl-3-(1-methylethyl)**		**2H-Pyran, 2-(2,5-hexadiynyloxy) tetrahydro**		**Octanal**		**Glycerin**	
Formula	$C_{11}H_{20}O_4$		$C_6H_8N_2O_8$		$C_{12}H_{18}O$		$C_9H_{14}O_2$		$C_8H_{16}O$		$C_3H_8O_3$	
Conc / Type Of biscuits	**RT**	**Area Sum %**	**RT**	**Area Sum %**	**RT**	**Area Sum %**	**RT**	**Area Sum %**	**RT**	**Area Sum %**	**RT**	**Area Sum %**
Barley biscuit	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Wheat biscuit	4.501	0.55	4.614	0.36	4.804	0.33	ND	ND	ND	ND	ND	ND
White maize biscuit	4.516	0.18	4.614	0.11	4.796	0.13	2.36	0.3	2.413	0.55	3.787	2.09
Sorghum biscuit	4.515	0.6	4.607	0.31	ND	ND	ND	ND	2.406	0.66	3.779	2.32
Oats biscuit	4.516	0.14	ND	ND	ND	ND	2.36	1.18	ND	ND	ND	ND

RT: retention time

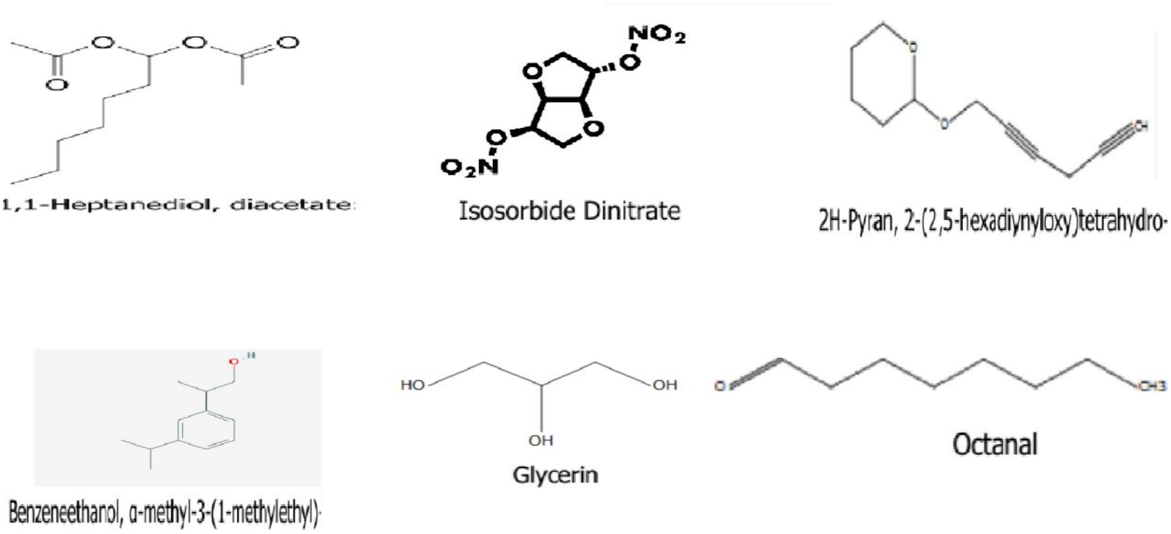


Fig. 3. Chemical structures of minor compounds in infested biscuits which were separated by GC-MS

Sensory evaluation of biscuits

The sensory evaluation of biscuits prepared by different types of infested flour (barley, wheat, White maize, sorghum and oats) present in Table, 4. It can be seen from this table that biscuits from barley had the lowest sensory evaluation of taste (1.17) and odour (0.833) characteristics, followed by biscuits from wheat, White maize, sorghum, and then oats. It was because the adults' cumulative number in barley flour was the highest (646 adults) as shown from Table 1, as well as the highest percentage of benzoquinone in barley biscuit (11.10 mg / g). The texture characteristic had rather good values. While the color and the overall acceptability characteristics of the biscuit samples from barley had the lowest values, which are 3.67 and 0.83 respectively, followed by the biscuit samples from wheat, White maize, sorghum and oats. Maybe this is due to its distinctive taste and smell of quinones.

Table 4. Sensory quality of biscuits prepared by different types of infested flour

Parameters / Biscuits	Color (10)	Taste (10)	Texture (10)	Odour (10)	Overall acceptability (10)
Barley	$3.67^{c} \pm 0.17$	$1.17^{d} \pm 0.17$	$6.5^{cd} \pm 0.29$	$0.833^{d} \pm 0.17$	$0.83^{d} \pm 0.17$
Wheat	$7.67^{a} \pm 0.33$	$1.83^{c} \pm 0.17$	$9.17^{a} \pm 0.17$	$1.67^{c} \pm 0.17$	$2.83^{c} \pm 0.17$
White maize	$7.33^{a} \pm 0.33$	$2.17^{c} \pm 0.17$	$6.17^{d} \pm 0.17$	$2.17^{c} \pm 0.17$	$3.17^{c} \pm 0.17$
Sorghum	$5.33^{b} \pm 0.33$	$3.5^{b} \pm 0.28$	$7.17^{b} \pm 0.17$	$3.67^{b} \pm 0.17$	$4.17^{b} \pm 0.17$
Oats	$4.67^{b} \pm 0.33$	$4.33^{a} \pm 0.17$	$6.83^{bc} \pm 0.17$	$4.5^{a} \pm 0.29$	$5.5^{a} \pm 0.29$
Duncan "F" test	**31.41****	**42.71****	**35.71****	**57.35****	**76.57****

Also, the values of titrated acidity in all types of infested flour were high, especially barley flour, the highest value was (137.12%), as reported by **Salwa Pasha and Samah Abdel-Tawab (2022)**. In contrast to biscuits made from barley, biscuits made from oats had a somewhat overall acceptability acceptance compared to the other of the types

Our results agree with **Smith et al. (1971)** mentioned that quinone secretions have a negative impact on the baking and savour qualities of bread prepared from infested flour by *Tribolium confusum*, causing many changes such as a progressive blackening of the crumb, a decrease in slice size, and a distinct offensive taste and odour. **Özkaya et al. (2009**) it was also confirmed by that bread made from hard and soft wheat that had previously been infested with the confused flour beetle, *Tribolium confusum,* showed a decrease in loaf volume as well as a very unpleasant taste and smell after 4 months of infestation.

4. CONCLUSIONS:

It could be concluded that the baking process had no effect on removing the previously secreted benzoquinones in the flour used to make the biscuits. On the contrary, the risk increases as the benzoquinone compound is exposed to cracking and the formation of more dangerous organic compounds. On the other hand, the Sensory characteristics of all types of biscuits were rated as poor.

ACKNOWLEDGMENT :

We thank Dr. Ahmed R. Khalaf, Food Science and Technology Dept., National Research Center, Dokki, Giza, Egypt, for his efforts in analysing samples. Dr. Gehad M. Khattab, Central Agricultural of Pesticides Laboratory, Agriculture Research Center, Dokki, Giza, Egypt, provide us with the standard material.

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الملخص العربي

تأثير عملية الخبيز على البنزوكينونات المفرزة بواسطة خنافس الدقيق المتشابهة *Tribolium confusum* (Du Val) فى البسكويت المصنوع من أنواع مختلفة من الدقيق المصاب

أجريت التجربة لتقدير مستويات البنزوكينون في البسكويت المخبوز على حرارة ١٨٠ درجة مئوية لمدة ٢٠ دقيقة بخمسة أنواع مختلفة من الطحين: دقيق القمح، الشعير، الذرة الرفيعة، الذرة الرفيعة والشوفان، المصابه بخنفساء الطحين المتشابهة، *Tribolium confusum* والمخزنة لمدة ثلاثة أشهر. أظهرت النتائج وجود مستويات عالية من البنزوكينون في جميع أنواع البسكويت. سجل بسكويت الشعير أعلى نسبة عند ١١.١٠ ملجم / جم ، يليه القمح ٦.٢٧ ملجم / جم ، والذرة البيضاء ٥.١٠ ملجم / جم ، والذرة الرفيعة ٢.٧٢ ملجم / جم ، والشوفان ٠.٩٥ ملجم / جم ، على التوالي. كما أظهرت البيانات وجود علاقة ارتباط موجبة بين العدد الكلي للحشرات في الدقيق المصاب قبل الخبز ومستوى البنزوكوينون بعد الخبز. نتج عن تكسير البنزوكينون بفعل حرارة الفرن أثناء خبز البسكويت العديد من المركبات العضوية الضارة والتي تم تحديدها من خلال تحليلها على .GC / MS . جميع المركبات الرئيسية كانت موجودة بنسب مرتفعة في جميع عينات البسكويت ، وهي: ٤،٤.١-تريميثيل سيكلوهيكسا-٢-أون-١-أول ، والتي تمثل أعلى نسبة من المركبات المنفصلة ، تراوحت بين ٤١.٩٥-٥٠.٩٢٪ ، ٢-أوكتينال ٢-بيوتيل والتي مثلت بنسبة ٢١.٨٥ - ٢٣.٦٤٪. ٧Octen-4-one-، 2،6-dimethyl تمثل نسبة ١٢.٥٥-١٧.٥٤٪ ؛ حمض الفاليريك ٤-ترايدسيل إستر ويتمثل بنسبه ٦.٢٥-٩.١٤٪ ، كانت النسبة المئوية لمساحة الفينول ٣.٥٣-٧.٤٨٪ وكانت النسبة المئوية لمساحة إستر فينيل حمض الكارباميك ٠.٥٥ - ٣.٠٣٪. بالإضافة إلى ٦ مركبات ثانوية أخرى. خلصنا إلى أن البسكويت المصنوع من الدقيق المصاب بالغ الخطورة بسبب وجود مادة البنزوكوينون والعديد من المركبات العضوية الأخرى الناتجة عن التكسير الحراري. كما أظهر التقييم الحسي للبسكويت المصنوع من الدقيق المصاب طعمًا ورائحة غير مرغوب فيها وداكنة في اللون. يعرضنا الغش التجاري لنفس المخاطر من خلال الجمع بين الدقيق أو النخالة المصاب بالطحين غير المصاب.

الكلمات المفتاحية: بنزوكينون ، الهدم الحراري ، بسكويت مصاب، *Tribolium confusum* ، HPLC ، GC-Ms.