



## Exploring Lipid fraction, Aflatoxin Levels and Shelf Life in Industrial Biscuits

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### Abstract

This study examined the presence of aflatoxins, fatty acid compositions, and storage stability in cookies (BSC), digestive biscuits (BSD), and crackers (BScr) produced in different countries. The findings showed that AFB2 and AFG2 were not found in the studied biscuits, but AFB1 was detected in BSD1-S, BSD1-I, BSC2-U, and BScr1-T, while AFG1 was only found in BSD1-S and BScr1-T. The saturated fatty acid content in BSC, BSD, and BScr samples varied from 20.69% to 45.90%, 10.90% to 47.03%, and 11.35% to 46.88%, respectively. The unsaturated fatty acid content in BSC, BSD, and BScr samples ranged from 11.31% to 59.17%, 12.40% to 74.24%, and from <LOD to 68.79%, respectively. The peroxide value, a deterioration index, ranged between 0.23 and 2.25 mEqO<sub>2</sub>/kg the day when biscuits were prepared but increased constantly and rapidly with storage and after 90 days it ranged between 9.03 and 11.05 mEqO<sub>2</sub>/kg. The level of HMF is a freshness index, the lower the value, the higher the freshness; in all the studied biscuits it was between 4.69 (at T<sub>0</sub>) and 178.33 mg/kg after 90 days. When prepared, only 2 out of the 36 tested biscuits were above the microbial limit of 4 log cfu/g whereas after 90 days, only 7 samples were below this



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limit. This study provides an overview of aflatoxin content in biscuits and highlights the importance of limiting the intake of saturated and trans fats for health-conscious consumers.

## Introduction

Palatability, affordability, long shelf life and easy availability and packing for masses of people make biscuit a popular processed food product around the world. It is possible to incorporate a wide range of nutrients and functionalities into biscuits because they have a variety of ingredients including flour, fat, sugar, water, milk, salt, fruit industry by-product and artificial yeast as raising agents.<sup>1-9</sup>

The Peroxide value was used by other authors as a deterioration index and previous findings evidenced an increase with storage in biscuits prepared with *Vigna mungo* flour<sup>1</sup> and with *Lagenaria siceraria* flour.<sup>2</sup> The moisture content facilitates the growth of microorganisms and for this reason its increase is negatively considered but previous findings showed that this parameter increases with storage.<sup>6</sup> Recent decades have seen increased public concern about food safety, notably agricultural contamination. Each year, mycotoxins destroy 25–50% of agricultural goods in the world, primarily in underdeveloped tropical nations, according to the FAO.<sup>10</sup> Mycotoxins in these items affects public health and the worldwide commerce of commodities. Cereals and cereal-derived products are especially sensitive to fungus and mycotoxin contamination. Wheat, one of the most popular grains, provides more nutrients than any other food item. However, wheat flour is prone to aflatoxins (AFs) contamination. AFs are produced from *Aspergillus flavus*, *A. parasiticus*, and *A. nominus*. They can cause cancer in humans and animals.<sup>11</sup> These mycotoxins can enter the human food chain by the ingestion of infected grain or processed foods like meat or other animal products fed contaminated food. Although fungal growth and aflatoxin synthesis can be prevented, mycotoxins in food cannot be controlled.<sup>12</sup> AFs consists of B1, B2, G1, G2, M1, and M2, in which AFB1 is the potent hepatocarcinogen.<sup>13</sup> AFB1 was classified as Group 1 carcinogen to humans by the International Agency for Research on Cancer (IARC).<sup>14</sup> For human health, the European Commission (EC) has set the maximum AFB1 level of 2–8 µg/kg for AFB1 and 15 µg/kg for total AFs in foods.<sup>15</sup> The warm-humid climate is good for *Aspergillus flavus*

and aflatoxin formation. Prevalence of AFB1 in the food chain, mainly in bakery products prepared with wheat flour, can cause hepatotoxicity, teratogenicity, immunosuppressive conditions and carcinogenicity.<sup>16</sup> Wheat and wheat-derived products are very important in the human diet and consequently there is pressing concern that mycotoxins originated in food raw materials may contaminate terminal bakery products. The increasing consumption of products prepared with wheat has caused an increasing attention towards mycotoxin prevalence and destiny in bakery processing. However, there are few data on AFs occurrence in baked food, including biscuits.

Additionally, the production of commercial biscuits utilizes animal fat and vegetable shortenings made from hydrogenated oils as fat ingredient. For example, vanaspati, a partially hydrogenated vegetable oil, is used for baking and frying in India, which is due to its semi-solid state at room temperature.<sup>17</sup> The formation of trans fatty acid (TFA) from cis-unsaturated fatty acids can be due by the partial hydrogenation and thermal treatment of vegetable oils. It has been well known that TFA is associated with cardiovascular disease for its intake. As reported previously, TFA raises low-density lipoprotein cholesterol and decreases high-density lipoprotein cholesterol, which may lead to proinflammatory alterations, endothelial dysfunction, and perhaps insulin resistance as well as displacement of important fatty acids from membranes.<sup>18</sup> The 2019 Global Burden of Disease Study showed that more than 600,000 annual deaths worldwide occurred due to high TFA intakes.<sup>19-20</sup> USA, Canada, and EU have required voluntary TFA labeling rules.<sup>17</sup> Denmark limits TFA to 2% in cooking oils and fats.<sup>19</sup> The United States Food and Drug Administration (USFDA) requires a statement related to TFA content in grams per serving right below the content of saturated fat on food labels. Foods containing low TFA (less than 0.5 g) per serving may claim zero trans fats or not a significant source on the label.<sup>17</sup> The Food Safety Standards Authority of India (FSSAI) recommends that TFA in partly hydrogenated vegetable oils (vanaspati) cannot exceed 10% of total fatty acid content.<sup>21</sup> The

World Health Organization (WHO) recommends less than 1% of energy from TFAs in the human diet.<sup>22</sup>

Therefore, the current study aimed to investigate AF contents and fatty acid composition in some commercial brands of biscuits collected from four countries (Spain, Iran, UAE, and Turkey). Furthermore, the effects of storage periods (at room temperature) of biscuit samples on quality parameters, including moisture content, peroxide value, HMF and microbiological analysis were analyzed for shelf-life evaluation.

## Materials and Methods

### Sample Collection

In Basrah, Iraq, retail shops and supermarkets sold nine biscuit brands: BSC<sub>1</sub>, BSC<sub>2</sub>, BSC<sub>3</sub>, BSD<sub>1</sub>, BSD<sub>2</sub>, BSD<sub>3</sub>, BScr<sub>1</sub>, BScr<sub>2</sub>, and BScr<sub>3</sub>. All these biscuits came from Spain, Iran, UAE, and Turkey. Three types of biscuits were randomly sampled: cookies (BSC), digestive biscuits (BSD), and crackers (BScr). These samples were chosen to represent popular brands consumed by different income ranges and age groups, especially children. It was also affected by biscuit availability during the research.

### Extraction and Detection of Aflatoxins in Biscuit Samples

AFB1, AFB2, AFG1, and AFG2 were extracted from biscuit samples according to<sup>23</sup> with some modifications. 5 g of dried and grinded biscuits was weighed into a beaker of 250 mL with 20 mL of a solution (water/acetonitrile, 16:18, v/v). Then it was placed on the vibrator (Sheaker) for 90 min for homogenization. The filtration was conducted with a paper Whatman No. 4 filter, before to concentrate to 2-5 mL with a rotary evaporator (50°C). The determination of AFs was performed by using high-performance liquid chromatography (HPLC) (Sykam GmbH, Gilching, Germany) with UV-visible detection. A mixture of acetonitrile:distilled water (70:30) was used as a mobile phase and 0.7 mL/min as flow rate. AFs was separated on a C18 – ODS Hypersil column (250 mm length × 4.6 mm I.D., 5 µm particle size, Maisch, Ammerbuch, Germany), the wavelength was set at 440 nm.

### Fatty Acid Analysis by Gaschromatography-Mass Spectrometry (GC-MS)

The esterification of fatty acids was conducted in a test tube in which were introduced and homogenized

0.5 mL of the extracted fat and 2 mL of ethylic alcohol (1 min). At this point were added respectively: 0.5 mL sulfuric acid and the mixture was cooled; n-hexane (2 mL) and deionized water (2 mL), then the mixture was carefully shaken (30 s) to react until different layers were obtained. The so prepared fatty acid methyl esters (FAMEs) were analysed by GC-MS technique.<sup>24</sup> An Agilent 7890 GC and an Agilent 5975C inert XC MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) were used. The system was equipped with a HP-5 MS capillary column (30 m length × 0.32 mm internal diameter × 0.25 µm film thickness). FAMEs were analysed by a splitless injection. The initial GC oven temperature was 40°C (5 min), then it was raised to 300°C (10°C/min). The generated mass spectra were matched with the NIST14 Mass Spectral library for the identification of fatty acids.

### Determination of Moisture

The biscuits were powdered after that they were dried in an oven at 104–105°C until constant weight.<sup>25</sup>

### Determination of Peroxide Value

The method proposed by Liu *et al.*<sup>26</sup> was applied. Five grams of sample were combined with a 30 mL acetic acid-chloroform solution, in a 20 mL: 10 mL ratio, in a conical flask and spun to dissolve. 0.5 mL potassium iodide (saturated solution) was added and titrated with 0.01 N sodium thiosulfate until the yellow tint was practically gone. Six-seven drops of starch solution (1% in deionized water) were added and the titration continued to release all iodine from the chloroform layer until the blue color disappeared. At the same time a blank analysis was conducted. The peroxide value was expressed as mL equivalent peroxide/kg sample.

### Determination of HMF

HMF in biscuits samples was determined based on Švecová and Mojmír.<sup>27</sup> In a 100 mL volumetric flask, 3 g of powdered and homogenized material and 50 mL of deionized water were ultrasonicated for 15 minutes to extract HMF. Then, 1 mL of Carrez II (ZnSO<sub>4</sub> 20%) and 1 mL of Carrez I (K<sub>4</sub>[Fe(CN)<sub>6</sub>] 15%) were added and deionized water was added to 100 mL HMF was evaluated using an Agilent Technologies HPLC system with a 285 nm UV/VIS detector. Sample aliquots of 25 µm were manually injected. Use of Agilent Zorbax Reliance Cartridge

guard column and Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm). For isocratic elution, the mobile phase was 90:10 water/acetonitrile. The analytical period was 10 min, and HMF was eluted at 2.7 min.

### Microbiological Analysis

Total counts of bacteria were determined by using Nutrient agar. Samples were cultured in nutrient agar and incubated at 37°C (48 h), then colonies of total bacteria count were counted as colony forming units (CFU) per mL.<sup>28</sup>

### Statistical Analysis

Analyses were conducted in triplicate, and data were presented as means ± standard deviations. The study used SPSS 13 software (SPSS Inc.,

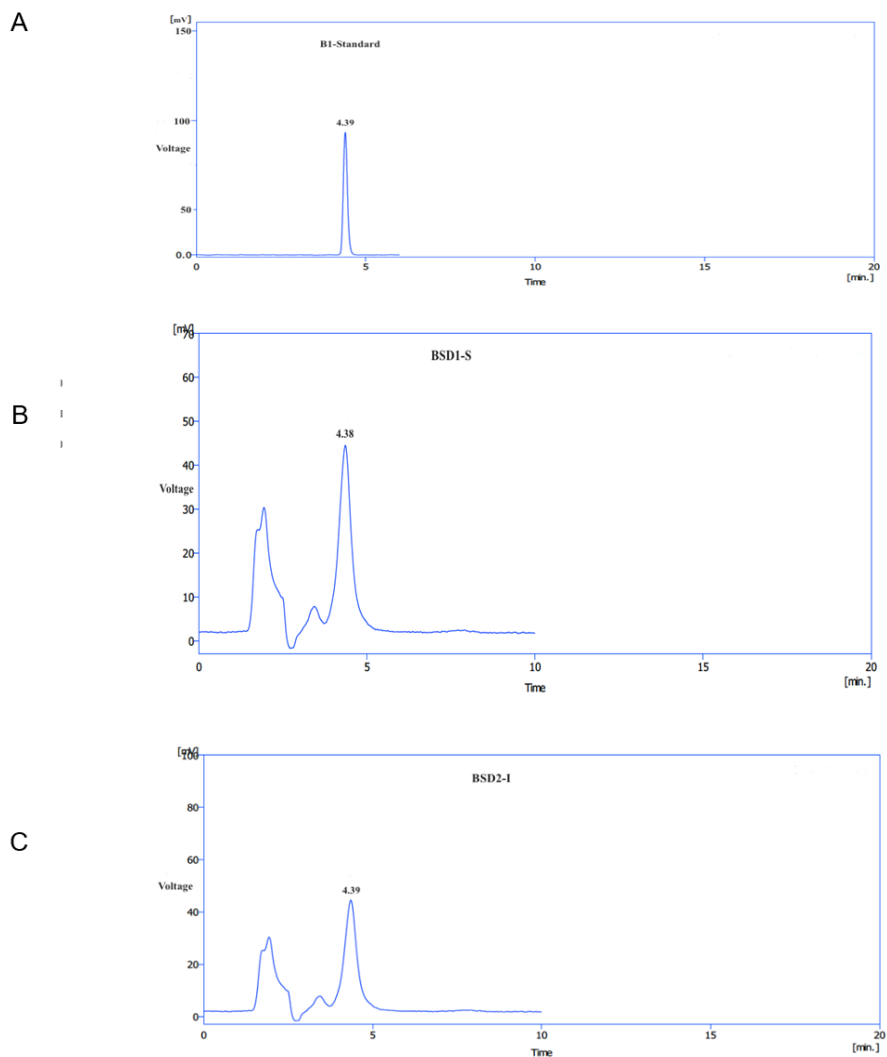
Chicago, IL, USA) to analyze the results using one-way analysis of variance (ANOVA) and the LSD test to compare treatments where significant ( $p < 0.05$ ).

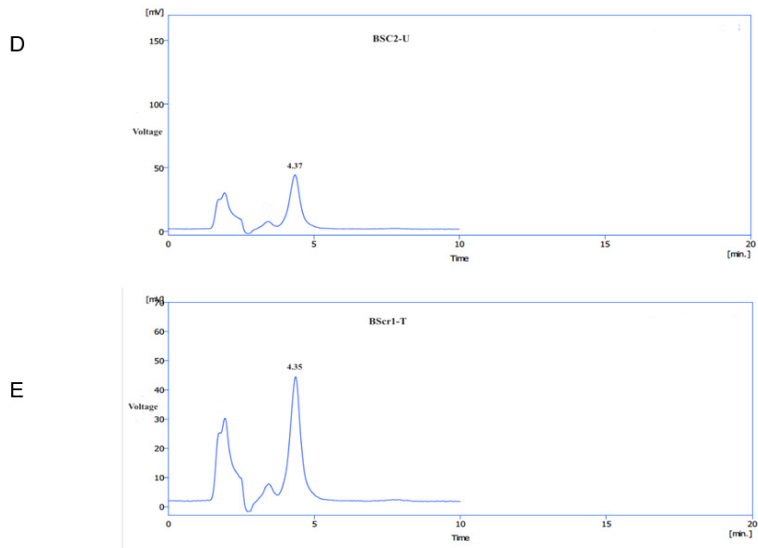
### Results and Discussion

#### Aflatoxines in Biscuit Samples Manufactured in Spain, Iran, UAE and Turkey

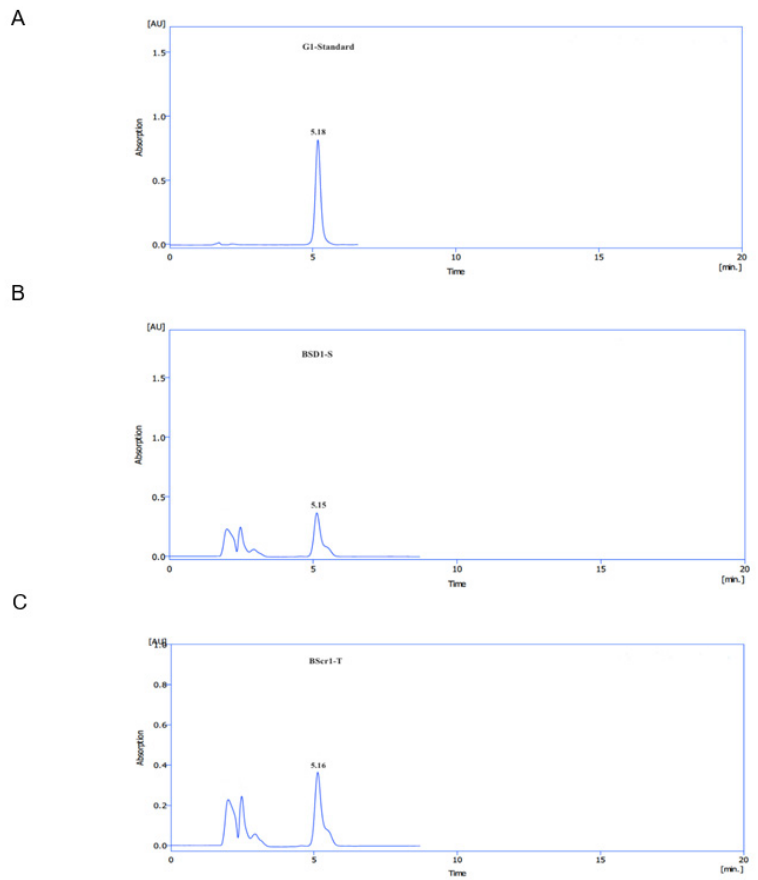
Figures 1 and 2 depict the HPLC chromatograms for AF standards and biscuit samples made in Spain, Iran, UAE and Turkey.

The AFB1, AFB2, AFG1, and AFG2 were analyzed in all tested samples, and the results are summarized in Table 1. This analytical method was applied because it demonstrated to be effective in the analysis, detection and quantification of Aflatoxines.





**Fig. 1: HPLC chromatograms of B1 standard (A), BSD1-S sample (B), BSD1-I sample (C), BSC2-U sample (D), and BScr1-T sample (E)**



**Fig. 2: HPLC chromatogram of G1 standard (A), BSD1-S sample (B), and BScr1-T sample (C)**

**Table 1: Aflatoxines contents in analyzed biscuits samples. Data represent means  $\pm$  standard deviation (n=3). ND: not detected**

Biscuit samples	AFs ( $\mu\text{g} / \text{kg}$ )				
	AFB1	AFB2	AFG1	AFG2	Total
BSD <sub>1</sub> -S	42.62 $\pm$ 1.05	ND	8.99 $\pm$ 0.73	ND	51.61 $\pm$ 1.24
BSD <sub>2</sub> -I	18.97 $\pm$ 0.95	ND	ND	ND	18.97 $\pm$ 0.95
BSC <sub>2</sub> -U	11.30 $\pm$ 1.26	ND	ND	ND	11.30 $\pm$ 1.26
BScr <sub>1</sub> -T	56.24 $\pm$ 2.09	ND	12.07 $\pm$ 1.34	ND	68.31 $\pm$ 1.49

From Figures 1 and 2, only BSD<sub>1</sub>-S, BSD<sub>1</sub>-I, BSC<sub>2</sub>-U, and BScr<sub>1</sub>-T contained detectable AFs, in which AFB<sub>2</sub> and AFG<sub>2</sub> were not found in all tested biscuits samples regardless of manufacturing country. However, AFB<sub>1</sub> was found in only four of 36 tested samples, that is, BSD<sub>1</sub>-S, BSD<sub>1</sub>-I, BSC<sub>2</sub>-U, and BScr<sub>1</sub>-T. Furthermore, AFB<sub>1</sub> contents in BScr<sub>1</sub>-T (56.24  $\mu\text{g}/\text{kg}$ ) and BSD<sub>1</sub>-S (42.62  $\mu\text{g}/\text{kg}$ ) were significantly higher than that in BSD<sub>2</sub>-I (18.97  $\mu\text{g}/\text{kg}$ ) and BSC<sub>2</sub>-U (11.30  $\mu\text{g}/\text{kg}$ ). Additionally, it was also observed that AFG<sub>1</sub> was only detected in BSD<sub>1</sub>-S (8.99  $\mu\text{g}/\text{kg}$ ) and BScr<sub>1</sub>-T (12.07  $\mu\text{g}/\text{kg}$ ). This finding was in agreement with Zain<sup>29</sup> who mentioned that the presence of AFs might be due to the secondary metabolism of some filamentous fungi under some suitable conditions such as temperature and humidity. The Codex Alimentarius Commission was considering a recommendation to

establish a maximum limit of 15 ppb for total AFs in foods worldwide.<sup>30</sup> Therefore, to ensure the dietary health of consumers, it is worth paying attention to improving the manufacturing technique of some biscuit samples, such as BScr<sub>1</sub>-T, BSD<sub>1</sub>-S, and BSD<sub>2</sub>-I. Gholamour Azizi and Rohui<sup>31</sup> in a study in North Iran, found less than 4 ppb in the 86.7% and more than 4 ppb in the 13.3% of the studied biscuits and cookies showing the large part of these bakery products within the International qualitative limits in Aflatoxines.

#### Fatty Acid Composition in Biscuit Samples Manufactured in Spain, Iran, Uae and Turkey

The GC-MS analysis was conducted to have very detailed results. In all tested biscuit samples, palmitic (C16:0) and oleic (C18:1) acids were found to most represented in many biscuit samples (Table 2).

**Table 2: Major fatty acids in tested biscuits manufactured in Spain, Iran, UAE and Turkey. Data represent means  $\pm$  standard deviation (n=3). ND: not detected. NA: not applicable**

Biscuit samples	Fatty acids (% of total FAs)						
	C <sub>16:0</sub>	C <sub>18:0</sub>	C <sub>18:1</sub>	C <sub>18:2</sub> (PUFA)	SFA	PUFA/SFA	TFA
BSC <sub>1</sub> -S	19.03 $\pm$ 0.58	2.74 $\pm$ 0.06	ND	11.31 $\pm$ 0.53	21.78 $\pm$ 0.39	0.52	21.43 $\pm$ 0.08
BSC <sub>2</sub> -S	19.20 $\pm$ 0.32	ND	55.22 $\pm$ 0.45	ND	20.89 $\pm$ 0.47	NA	ND
BSC <sub>3</sub> -S	14.23 $\pm$ 0.18	0.64 $\pm$ 0.05	59.17 $\pm$ 0.19	ND	20.69 $\pm$ 0.11	NA	ND
BSC <sub>1</sub> -I	29.71 $\pm$ 0.26	2.59 $\pm$ 0.08	ND	9.56 $\pm$ 0.08	36.92 $\pm$ 0.37	0.26	ND
BSC <sub>2</sub> -I	31.11 $\pm$ 0.13	ND	47.14 $\pm$ 0.26	ND	32.37 $\pm$ 0.21	NA	11.95 $\pm$ 0.06
BSC <sub>3</sub> -I	29.59 $\pm$ 0.08	0.61 $\pm$ 0.17	ND	6.27 $\pm$ 0.07	37.66 $\pm$ 0.34	0.17	ND
BSC <sub>1</sub> -U	35.88 $\pm$ 0.13	0.55 $\pm$ 0.05	45.31 $\pm$ 0.37	ND	36.42 $\pm$ 0.18	NA	6.47 $\pm$ 0.02
BSC <sub>2</sub> -U	17.75 $\pm$ 0.14	4.02 $\pm$ 0.09	ND	2.33 $\pm$ 0.01	21.77 $\pm$ 0.37	0.11	1.49 $\pm$ 0.01
BSC <sub>3</sub> -U	38.14 $\pm$ 0.51	0.63 $\pm$ 0.04	27.99 $\pm$ 0.22	7.34 $\pm$ 0.03	40.26 $\pm$ 0.42	0.18	ND
BSC <sub>1</sub> -T	31.84 $\pm$ 0.83	1.23 $\pm$ 0.03	ND	15.39 $\pm$ 0.09	34.45 $\pm$ 0.91	0.45	11.94 $\pm$ 0.07
BSC <sub>2</sub> -T	41.49 $\pm$ 0.72	0.56 $\pm$ 0.01	ND	34.78 $\pm$ 0.53	45.90 $\pm$ 0.80	0.76	5.89 $\pm$ 0.04
BSC <sub>3</sub> -T	37.60 $\pm$ 0.28	0.51 $\pm$ 0.02	28.44 $\pm$ 0.18	12.49 $\pm$ 0.12	39.58 $\pm$ 0.31	0.32	ND

Mean	28.80	1.17	21.94	8.29	32.39		4.93
BSD <sub>1</sub> -S	10.80 ± 0.07	ND	66.32 ± 1.08	ND	19.13 ± 0.13	NA	ND
BSD <sub>2</sub> -S	10.53 ± 0.37	ND	68.79 ± 0.73	ND	11.35 ± 0.18	NA	4.53 ± 0.09
BSD <sub>3</sub> -S	13.27 ± 0.09	0.85 ± 0.09	47.51 ± 0.86	9.49 ± 0.41	22.52 ± 0.13	0.42	ND
BSD <sub>1</sub> -I	28.20 ± 0.21	11.46 ± 0.12	ND	20.09 ± 0.05	41.78 ± 0.29	0.48	ND
BSD <sub>2</sub> -I	29.28 ± 0.19	0.62 ± 0.08	ND	40.98 ± 0.24	30.33 ± 0.31	1.35	ND
BSD <sub>3</sub> -I	37.45 ± 0.28	0.66 ± 0.02	ND	ND	41.19 ± 0.40	NA	4.67 ± 0.04
BSD <sub>1</sub> -U	40.98 ± 0.26	0.73 ± 0.04	ND	8.90 ± 0.05	45.50 ± 0.28	0.20	ND
BSD <sub>2</sub> -U	38.08 ± 0.18	0.56 ± 0.03	46.26 ± 0.61	ND	39.41 ± 0.39	NA	ND
BSD <sub>3</sub> -U	37.19 ± 0.17	0.93 ± 0.03	7.43 ± 0.08	11.14 ± 0.04	39.29 ± 0.21	0.28	ND
BSD <sub>1</sub> -T	11.59 ± 0.11	ND	ND	67.34 ± 0.59	14.07 ± 0.27	4.76	3.73 ± 0.01
BSD <sub>2</sub> -T	38.90 ± 0.24	0.51 ± 0.04	26.33 ± 0.20	11.36 ± 0.29	46.87 ± 0.34	0.24	ND
BSD <sub>3</sub> -T	32.27 ± 0.19	ND	ND	48.71 ± 0.34	38.37 ± 0.35	1.27	3.23 ± 0.01
Mean	27.38	1.36	21.89	18.17	32.48		1.35
BScr <sub>1</sub> -S	10.98 ± 0.18	9.24 ± 0.14	7.59 ± 0.04	63.31 ± 0.51	26.49 ± 0.22	2.38	ND
BScr <sub>2</sub> -S	8.72 ± 0.11	ND	5.62 ± 0.03	68.62 ± 0.97	15.99 ± 0.24	4.35	6.01 ± 0.08
BScr <sub>3</sub> -S	ND	0.77 ± 0.07	36.71 ± 0.16	20.91 ± 0.11	10.90 ± 0.16	1.92	11.21 ± 0.04
BScr <sub>1</sub> -I	44.53 ± 0.79	0.56 ± 0.04	9.57 ± 0.09	8.24 ± 0.09	47.03 ± 0.81	0.18	ND
BScr <sub>2</sub> -I	23.47 ± 0.26	ND	ND	25.22 ± 0.11	24.71 ± 0.32	1.02	25.35 ± 0.09
BScr <sub>3</sub> -I	34.44 ± 0.37	ND	6.60 ± 0.07	44.00 ± 0.26	35.98 ± 0.29	1.22	3.60 ± 0.01
BScr <sub>1</sub> -U	42.34 ± 0.60	ND	3.34 ± 0.01	9.06 ± 0.06	44.33 ± 0.79	0.20	ND
BScr <sub>2</sub> -U	36.80 ± 0.42	0.82 ± 0.02	6.41 ± 0.03	9.97 ± 0.02	37.99 ± 0.51	0.26	ND
BScr <sub>3</sub> -U	43.79 ± 0.49	0.48 ± 0.01	3.88 ± 0.03	10.95 ± 0.06	45.71 ± 0.37	0.24	ND
BScr <sub>1</sub> -T	38.27 ± 0.23	ND	ND	49.18 ± 0.51	42.31 ± 0.31	1.16	0.65 ± 0.01
BScr <sub>2</sub> -T	37.24 ± 0.43	ND	ND	42.79 ± 0.86	39.33 ± 0.62	1.09	0.95 ± 0.04
BScr <sub>3</sub> -T	35.94 ± 0.78	ND	26.99 ± 0.14	16.14 ± 0.18	38.30 ± 0.88	0.42	3.41 ± 0.02
Mean	29.71	0.99	8.89	30.70	34.09		4.27

For different brands of biscuit samples (BSC, BSD, or BScr) manufactured in the same country, fatty acid profiles exhibited many differences in SFA, MUFA, PUFA, and TFA. For example, C16:0 content in BSC<sub>3</sub>-S (14.23%) was significantly lower than that in BSC<sub>1</sub>-S (19.03%) and BSC<sub>2</sub>-S (19.20%), while stearic acid (C18:0) content in BSC<sub>3</sub>-S (0.64%) was higher than that in BSC<sub>2</sub>-S which was actually not detected. Additionally, BSC<sub>2</sub>-S and BSC<sub>3</sub>-S contained high amounts of C18:1 (55.22% and 59.17%, respectively), which was not detected in BSC<sub>1</sub>-S. However, linoleic acid (C18:2) and TFA was only found in BSC<sub>1</sub>-S (11.31% and 21.43%, respectively). For the same type of biscuit samples manufactured in different countries, fatty acid profiles also exhibited some differences. C16:0 contents in BSC (14.23%-19.20%), BSD (10.53%-13.27%), and BScr (ND-10.98%) samples manufactured in Spain were significantly lower than that in the corresponding biscuit samples manufactured in Iran, UAE, and Turkey ( $p < 0.05$ ). Conversely, C18:1 contents in biscuit samples manufactured in Spain,

especially BSD-S (47.51%-68.79%), were much higher than that in samples manufactured in other countries. Even in BSD-I samples, C18:1 was not detected at all. C18:2, as unique detected PUFA and essential FA, was also found as high-amount FA in some biscuit samples, such as BScr<sub>2</sub>-S (68.62%), BSD<sub>1</sub>-T (67.34%), BScr<sub>1</sub>-S (63.31%). In addition, although C18:0 was detected widely in tested biscuit samples, the levels were generally lower than the levels of C16:0, C18:1, and C18:2. Furthermore, C18:0 was not detected in some biscuit samples, such as all BScr samples manufactured in Turkey. As reported previously, in New Zealand, C16:0 contents in biscuits were similar to our findings, ranging between 16.1% and 38.3%, whereas C18:0 was much lower and amounted to ND-0.3%.<sup>32</sup> Caponio *et al.*<sup>33</sup> confirmed that high amounts of C16:0 indicated the utilization of palm oil in the production of biscuit samples. For different types of biscuit sample regardless of manufacturing country, there was little difference for C16:0 and C18:0 contents among BSC, BSD, and BScr samples.

However, C18:1 contents in BSC and BSD (21.94% and 21.89%, respectively) were much higher than that in BScr (8.89%). Conversely, BScr had the highest C18:2 level (30.70%), followed by BSD (18.17%) and BSC (8.29%).

Table 3 also showed the contents of some other FAs in biscuit samples. It was well observed that the biscuits samples also contained short-chain

FAs ranging between C8:0 and C10:0, which can be explained with the possible use of milk fat in the recipe. Caprylic acid (C8:0) was found only in BScr<sub>3</sub>-S (0.52%), which was within the limit of Turkish biscuits prepared for children (0.2%-0.8%). Small amounts of capric acid (C10:0) was detected in BSC<sub>2</sub>-S and BScr<sub>3</sub>-S (0.66% and 0.46%, respectively) than that in Spanish biscuits (2.3%).<sup>34</sup>

**Table 3: Minor fatty acids in in all tested biscuits manufactured in Spain, Iran, UAE and Turkey. Data represent means  $\pm$  standard deviation (n=3). ND: not detected**

Biscuit samples	Fatty acid (% of total FAs)					
	C <sub>8:0</sub>	C <sub>10:0</sub>	C <sub>12:0</sub>	C <sub>14:0</sub>	C <sub>20:0</sub>	C <sub>22:0</sub>
BSC <sub>1</sub> -S	ND	ND	ND	ND	ND	ND
BSC <sub>2</sub> -S	ND	0.66 $\pm$ 0.07	1.02 $\pm$ 0.04	ND	ND	ND
BSC <sub>3</sub> -S	ND	ND	ND	ND	ND	0.72 $\pm$
BSC <sub>1</sub> -I	ND	ND	ND	ND	ND	ND
BSC <sub>2</sub> -I	ND	ND	ND	ND	ND	ND
BSC <sub>3</sub> -I	ND	ND	ND	ND	ND	ND
BSC <sub>1</sub> -U	ND	ND	ND	ND	ND	ND
BSC <sub>2</sub> -U	ND	ND	ND	ND	ND	ND
BSC <sub>3</sub> -U	ND	ND	1.48 $\pm$ 0.02	ND	ND	ND
BSC <sub>1</sub> -T	ND	ND	1.38 $\pm$ 0.03	ND	ND	ND
BSC <sub>2</sub> -T	ND	ND	2.50 $\pm$ 0.03	ND	ND	ND
BSC <sub>3</sub> -T	ND	ND	0.54 $\pm$ 0.02	0.93 $\pm$ 0.01	ND	ND
Mean	ND	0.05	0.57	0.07	ND	0.06
BSD <sub>1</sub> -S	ND	ND	ND	ND	ND	1.44 $\pm$ 0.03
BSD <sub>2</sub> -S	ND	ND	ND	ND	ND	0
BSD <sub>3</sub> -S	ND	ND	ND	ND	ND	0.87 $\pm$ 0.01
BSD <sub>1</sub> -I	ND	ND	0.45	ND	ND	0
BSD <sub>2</sub> -I	ND	ND	ND	ND	ND	0.43 $\pm$ 0.02
BSD <sub>3</sub> -I	ND	ND	ND	ND	ND	ND
BSD <sub>1</sub> -U	ND	ND	ND	ND	ND	ND
BSD <sub>2</sub> -U	ND	ND	ND	ND	0.76	ND
BSD <sub>3</sub> -U	ND	ND	ND	ND	1.16	ND
BSD <sub>1</sub> -T	ND	ND	ND	ND	ND	1.78 $\pm$ 0.01
BSD <sub>2</sub> -T	ND	ND	2.69 $\pm$ 0.08	1.54 $\pm$ 0.02	ND	ND
BSD <sub>3</sub> -T	ND	ND	ND	0.93	ND	ND
Mean	ND	ND	0.26	0.20	0.16	0.37
BScr <sub>1</sub> -S	ND	ND	ND	ND	ND	1.37
BScr <sub>2</sub> -S	ND	ND	ND	ND	ND	1.54
BScr <sub>3</sub> -S	0.52 $\pm$ 0.02	0.46 $\pm$ 0.06	ND	0.66 $\pm$ 0.04	ND	0.85 $\pm$ 0.03
BScr <sub>1</sub> -I	ND	ND	ND	ND	ND	ND
BScr <sub>2</sub> -I	ND	ND	ND	ND	ND	ND
BScr <sub>3</sub> -I	ND	ND	ND	ND	ND	1.01
BScr <sub>1</sub> -U	ND	ND	ND	0.69	ND	ND

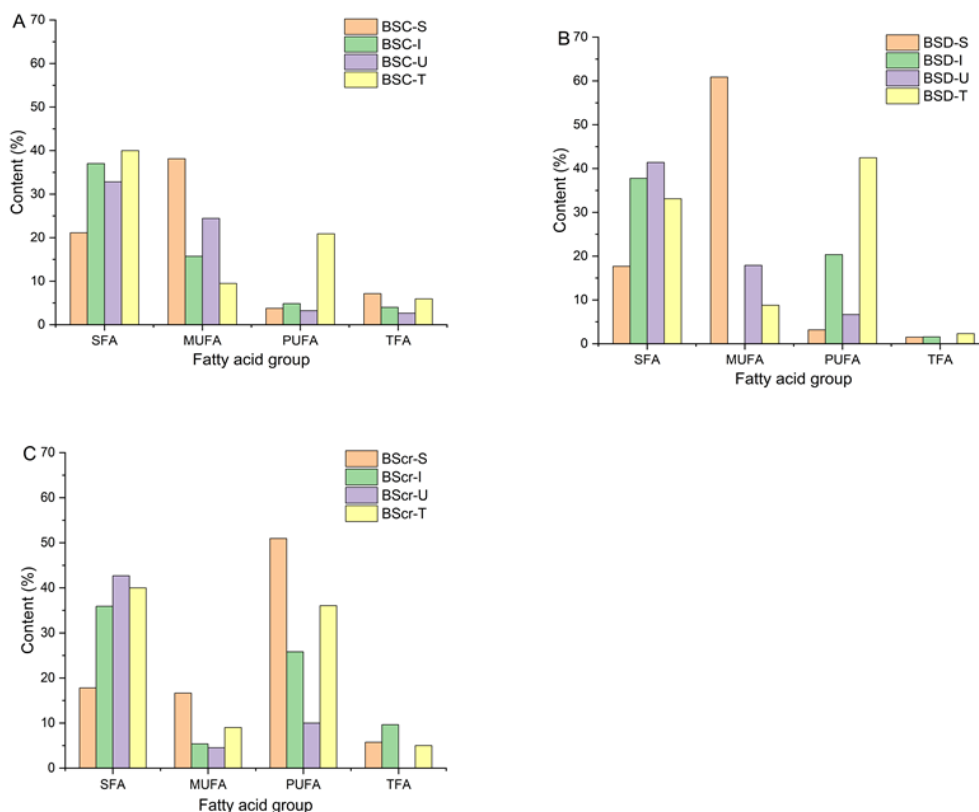


BScr <sub>2</sub> -U	ND	ND	0.36	ND	ND	ND
BScr <sub>3</sub> -U	ND	ND	ND	ND	ND	ND
BScr <sub>1</sub> -T	ND	ND	0.84	ND	ND	ND
BScr <sub>2</sub> -T	ND	ND	ND	ND	ND	0.50 ±
BScr <sub>3</sub> -T	ND	ND	ND	1.04	ND	0.62 ±
Mean	0.04	0.03	0.1	0.19	ND	0.49

The lauric acid (C12:0) content (0.36%-2.69%) was revealed in a small portion of biscuit samples in lower quantity if compared to Spanish biscuits (11.8%).<sup>34</sup> Myristic acid (C14:0) content (0.07%-1.54%) in some biscuit samples was comparable with New Zealand (0.1%-1.0%), Brazilian (0.10%-1.47%), and Turkish biscuits (0.6%-1.0%).<sup>35</sup> Arachidic acid (C20:0) were found only in BSD2-U (0.38%) and BSD3-U (0.37%), which was in agreement with that in Poland biscuits (0.35%-0.75%).<sup>36</sup> The contents of Behenic acid (C22:0) ranged from 0.43% to 1.54% in some analysed biscuits. This datum agrees with Chandana and Navaratne<sup>36</sup> who found the content of C22:0 was 0.77% in soft dough biscuits. It was

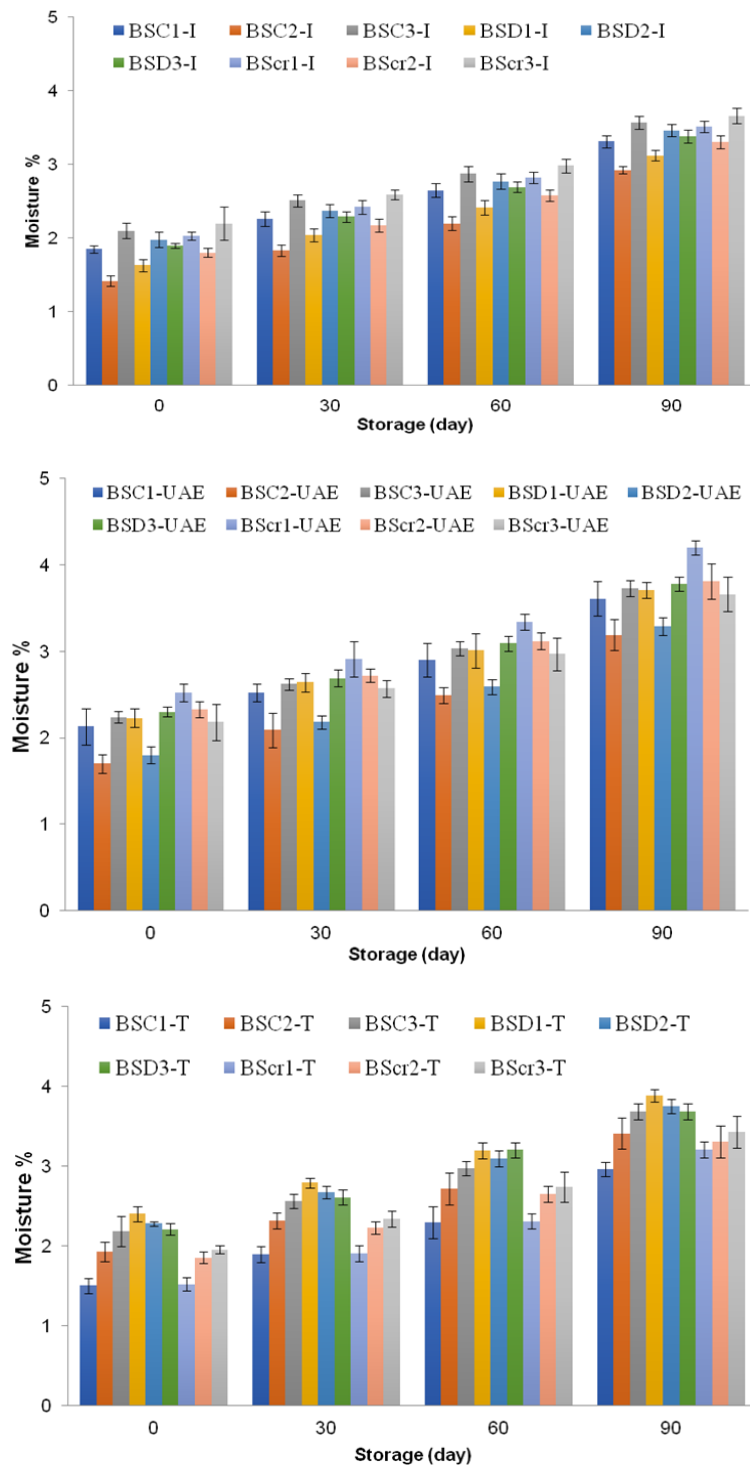
also observed that only BSC3-S contained C22:0 in BSC samples regardless of manufacturing country.

Figure 3 also shows the contents of FA groups including SFA, MUFA, PUFA, and TFA in biscuit samples from Spanish, Iran, UAE, and Turkey. From Figure 1 combining with Table 2, SFA contents in different brand of BSC, BSD, or BScr samples manufactured in the same region had more or less difference. For example, no significant difference in SFA contents among BSC<sub>1</sub>-S (21.78%), BSC<sub>2</sub>-S (20.89%), and BSC<sub>3</sub>-S (20.69%) was observed, but SFA content in BSC<sub>3</sub>-U (40.26%) was much higher than that in BSC<sub>2</sub>-U (21.77%).



**Fig. 3:** SFA, MUFA, PUFA, and TFA contents (% of total FA) in BSC (A), BSD (B), BScr (C) samples manufactured in Spain, Iran, UAE, and Turkey





**Fig. 4: Effect of storage periods on moisture content of Spanish, Iranian, UAE, and Turkish biscuit samples stored for 90 days at room temperature.**

The moisture contents of the Spanish biscuit samples were 3.43%, 3.56%, 3.52%, 3.67%, 3.41%, 3.55%, 3.52%, 3.55%, and 3.60% for BSC<sub>1</sub>-S, BSC<sub>2</sub>-S, BSC<sub>3</sub>-S, BSD<sub>1</sub>-S, BSD<sub>2</sub>-S, BSD<sub>3</sub>-S, BScr<sub>1</sub>-S, BScr<sub>2</sub>-S, and BScr<sub>3</sub>-S respectively for 90 days at room temperature. While the moisture contents in the Iranian biscuit samples were 3.31%, 2.92%, 3.57%, 3.12%, 3.46%, 3.38%, 3.51%, 3.30%, and 3.66% for BSC<sub>1</sub>-I, BSC<sub>2</sub>-I, BSC<sub>3</sub>-I, BSD<sub>1</sub>-I, BSD<sub>2</sub>-I, BSD<sub>3</sub>-I, BScr<sub>1</sub>-I, BScr<sub>2</sub>-I, and BScr<sub>3</sub>-I, respectively for 90 days at room temperature. For the UAE biscuit samples, the moisture contents were 3.61%, 3.19%, 3.73%, 3.71%, 3.29%, 3.78%, 4.20%, 3.81%, 3.66% for each of BSC<sub>1</sub>-U, BSC<sub>2</sub>-U, BSC<sub>3</sub>-U, BSD<sub>1</sub>-U, BSD<sub>2</sub>-U, BSD<sub>3</sub>-U, BScr<sub>1</sub>-U, BScr<sub>2</sub>-U, and BScr<sub>3</sub>-U, respectively when stored for 90 days at room temperature. The moisture content in the Turkish biscuit samples 2.96%, 3.41%, 3.68%, 3.88%, 3.75%, 3.68%, 3.20%, 3.31%, 3.43% for each of BSC<sub>1</sub>-T, BSC<sub>2</sub>-T, BSC<sub>3</sub>-T, BSD<sub>1</sub>-T, BSD<sub>2</sub>-T, BSD<sub>3</sub>-T, BScr<sub>1</sub>-T, BScr<sub>2</sub>-T, and BScr<sub>3</sub>-T, respectively, when stored for 90 days at room temperature.

The results of the statistical analysis showed that there was a significant effect ( $p < 0.05$ ) for each country of manufacture, types of biscuits and storage, and the interaction between the country of manufacture and the types of biscuits. The results

of the statistical analysis showed that there were no significant differences for the interaction between the types of biscuits and storage and the triple interference between the country of manufacture and the types of biscuits and storage. The results showed a gradual increase in the moisture content during the storage period in all the baked biscuit samples. This increase in the amount of moisture came as a result of absorbing all kinds of biscuits to the moisture from the environment surrounding the storage conditions. These results are consistent with Mahloko *et al.*<sup>43</sup> and McMinn *et al.*<sup>44</sup> who confirmed that the moisture content was within the recommended limits (0-10%) for stored biscuits. Similarly, Kumar *et al.* in biscuits prepared with multigrain flours confirmed the moisture increase at ambient temperature.<sup>45</sup>

#### Effect of Storage Periods on The Peroxide Value of Biscuit Samples Manufactured in Spain, Iran, Uae and Turkey

Peroxide value indicates the oxidative state of biscuits. A high peroxide value is the origin of unpleasant odors. A low peroxide value can be an index of a high storability. The results in Table 4 show the effect of storage periods on the value of peroxide in samples of Spanish, Iranian, UAE, and Turkish biscuits stored at room temperature for 90 days.

**Table 4: Effect of storage periods on the peroxide value of biscuit samples stored at room temperature for 90 days. Values express as mean  $\pm$  standard deviation of triplicate (n = 3).**

Biscuit sample	Storage (days)			
	0	30	60	90
BSC <sub>1</sub> -S	1.66 $\pm$ 0.05	3.06 $\pm$ 0.11	6.26 $\pm$ 0.37	10.46 $\pm$ 0.39
BSC <sub>2</sub> -S	1.75 $\pm$ 0.04	3.15 $\pm$ 0.19	6.35 $\pm$ 0.30	10.55 $\pm$ 0.10
BSC <sub>3</sub> -S	2.25 $\pm$ 0.07	3.65 $\pm$ 0.10	6.85 $\pm$ 0.33	11.05 $\pm$ 1.20
BSD <sub>1</sub> -S	1.00 $\pm$ 0.15	2.40 $\pm$ 0.11	5.60 $\pm$ 0.28	9.80 $\pm$ 0.58
BSD <sub>2</sub> -S	1.61 $\pm$ 0.10	3.01 $\pm$ 0.10	6.21 $\pm$ 0.30	10.41 $\pm$ 0.41
BSD <sub>3</sub> -S	0.69 $\pm$ 0.04	2.09 $\pm$ 0.10	5.29 $\pm$ 0.30	9.49 $\pm$ 0.03
BScr <sub>1</sub> -S	0.92 $\pm$ 0.04	2.32 $\pm$ 0.07	5.52 $\pm$ 0.28	9.72 $\pm$ 0.36
BScr <sub>2</sub> -S	1.50 $\pm$ 0.06	2.90 $\pm$ 0.11	6.10 $\pm$ 0.22	10.30 $\pm$ 0.21
BScr <sub>3</sub> -S	0.54 $\pm$ 0.03	1.94 $\pm$ 0.10	5.14 $\pm$ 0.21	9.34 $\pm$ 0.25
BSC <sub>1</sub> -I	1.41 $\pm$ 0.05	2.81 $\pm$ 0.10	6.01 $\pm$ 0.24	10.21 $\pm$ 0.11
BSC <sub>2</sub> -I	1.81 $\pm$ 0.07	3.21 $\pm$ 0.19	6.41 $\pm$ 0.27	10.61 $\pm$ 0.10
BSC <sub>3</sub> -I	0.55 $\pm$ 0.08	1.95 $\pm$ 0.09	5.15 $\pm$ 0.20	9.35 $\pm$ 0.20
BSD <sub>1</sub> -I	0.79 $\pm$ 0.10	2.19 $\pm$ 0.08	5.39 $\pm$ 0.24	9.59 $\pm$ 0.38
BSD <sub>2</sub> -I	0.83 $\pm$ 0.06	2.23 $\pm$ 0.09	5.43 $\pm$ 0.32	9.63 $\pm$ 0.18
BSD <sub>3</sub> -I	0.57 $\pm$ 0.03	1.97 $\pm$ 0.09	5.17 $\pm$ 0.28	9.37 $\pm$ 1.15

BScr <sub>1</sub> -I	0.25 ± 0.07	1.65 ± 0.04	4.85 ± 0.21	9.05 ± 0.10
BScr <sub>2</sub> -I	0.55 ± 0.05	1.95 ± 0.07	5.15 ± 0.28	9.35 ± 0.36
BScr <sub>3</sub> -I	0.54 ± 0.08	1.94 ± 0.07	5.14 ± 0.14	9.34 ± 0.26
BSC <sub>1</sub> -U	2.05 ± 0.07	3.45 ± 0.11	6.65 ± 0.26	10.85 ± 0.25
BSC <sub>2</sub> -U	0.77 ± 0.07	2.17 ± 0.10	5.37 ± 0.29	9.57 ± 0.24
BSC <sub>3</sub> -U	1.11 ± 0.09	2.51 ± 0.09	5.71 ± 0.29	9.91 ± 0.10
BSD <sub>1</sub> -U	0.94 ± 0.09	2.34 ± 0.10	5.54 ± 0.24	9.74 ± 0.06
BSD <sub>2</sub> -U	1.35 ± 0.07	2.75 ± 0.09	5.95 ± 0.24	10.15 ± 0.11
BSD <sub>3</sub> -U	1.21 ± 0.06	2.61 ± 0.087	5.81 ± 0.30	10.01 ± 0.18
BScr <sub>1</sub> -U	0.90 ± 0.05	2.30 ± 0.07	5.50 ± 0.31	9.70 ± 0.25
BScr <sub>2</sub> -U	1.04 ± 0.05	2.44 ± 0.05	5.64 ± 0.21	9.84 ± 0.09
BScr <sub>3</sub> -U	1.30 ± 0.09	2.70 ± 0.04	5.90 ± 0.30	10.10 ± 0.17
BSC <sub>1</sub> -T	0.45 ± 0.08	1.85 ± 0.04	5.05 ± 0.19	9.25 ± 0.08
BSC <sub>2</sub> -T	1.91 ± 0.09	3.31 ± 0.14	6.51 ± 0.39	10.71 ± 0.15
BSC <sub>3</sub> -T	2.15 ± 0.10	3.55 ± 0.11	6.75 ± 0.23	10.95 ± 1.05
BSD <sub>1</sub> -T	0.80 ± 0.05	2.20 ± 0.10	5.40 ± 0.21	9.60 ± 1.10
BSD <sub>2</sub> -T	0.57 ± 0.09	1.97 ± 0.09	5.17 ± 0.33	9.37 ± 0.19
BSD <sub>3</sub> -T	0.78 ± 0.06	2.18 ± 0.09	5.38 ± 0.18	9.58 ± 0.14
BScr <sub>1</sub> -T	0.46 ± 0.05	1.86 ± 0.08	5.06 ± 0.25	9.26 ± 0.05
BScr <sub>2</sub> -T	0.31 ± 0.03	1.71 ± 0.08	4.91 ± 0.28	9.11 ± 0.05
BScr <sub>3</sub> -T	0.23 ± 0.06	1.63 ± 0.06	4.83 ± 0.27	9.03 ± 0.11

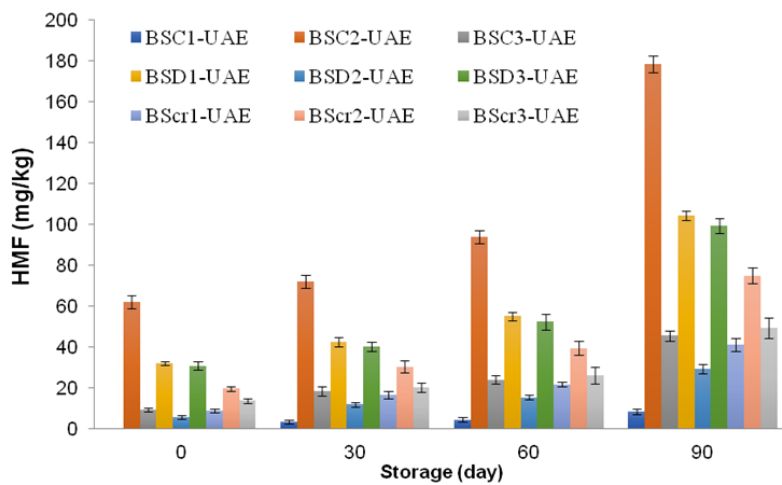
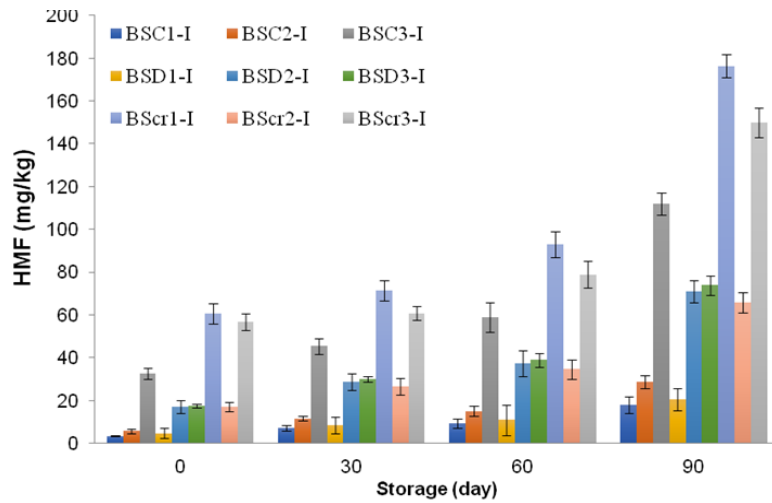
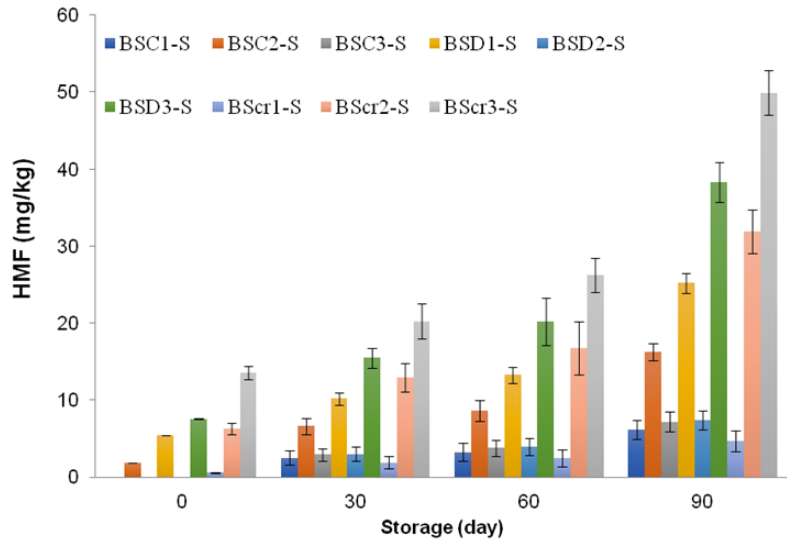
No significant differences for the interaction between the types of biscuits and storage and the triple interference between the country of manufacture and the types of biscuits and storage. The values of peroxide in the Spanish biscuit samples were 10.46, 10.55, 11.05, 9.8, 10.41, 9.48, 9.72, 10.30, 9.34 mEq/kg for each of BSC<sub>1</sub>-S, BSC<sub>2</sub>-S, BSC<sub>3</sub>-S, BSD<sub>1</sub>-S, and BSD<sub>2</sub>-S, BSD<sub>3</sub>-S, BScr<sub>1</sub>-S, BScr<sub>2</sub>-S, and S-BScr<sub>3</sub>, respectively. While the values of peroxide in the Iranian biscuit samples were 10.21, 10.61, 9.35, 9.59, 9.63, 9.37, 9.05, 9.35, 9.34 mEq/kg for BSC<sub>1</sub>-I, BSC<sub>2</sub>-I, BSC<sub>3</sub>-I, BSD<sub>1</sub>-I, BSD<sub>2</sub>-I, BSD<sub>3</sub>-I, BScr<sub>1</sub>-I, BScr<sub>2</sub>-I, and BScr<sub>3</sub>-I, respectively, stored for 90 days at room temperature. For the UAE biscuit samples, the peroxide values were 10.85, 9.57, 9.91, 9.74, 10.51, 10.01, 9.70, 9.84, 10.10 mEq/kg for each of BSC<sub>1</sub>-U, BSC<sub>2</sub>-U, BSC<sub>3</sub>-U, BSD<sub>1</sub>-U, BSD<sub>2</sub>-U, BSD<sub>3</sub>-U, BScr<sub>1</sub>-U, BScr<sub>2</sub>-U and BScr<sub>3</sub>-U, respectively. The values of peroxide in the Turkish biscuit samples ranged from 9.25, 10.71, 10.95, 9.60, 9.37, 9.58, 9.26, 9.11, 9.03 mEq/kg for each of BSC<sub>1</sub>-T, BSC<sub>2</sub>-T, BSC<sub>3</sub>-T, BSD<sub>1</sub>-T, BSD<sub>2</sub>-T, BSD<sub>3</sub>-T, BScr<sub>1</sub>-T, BScr<sub>2</sub>-T, and BScr<sub>3</sub>-T, respectively.

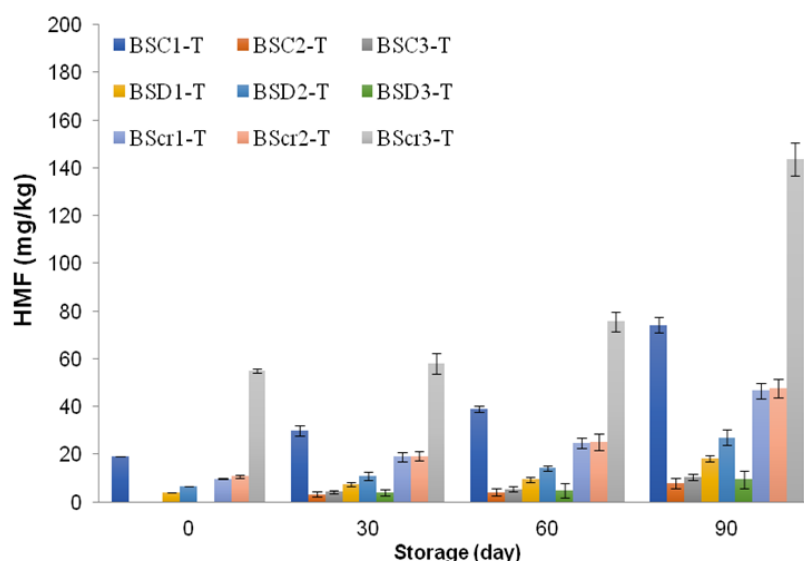
The results showed a gradual increase in the PV value during storage in all samples of baked biscuits. The results also showed that 13 of the total 36 types

of biscuits that were examined were higher than 10 mEq/kg, i.e. the permissible limit specified by the World Food Program (FAO, 2021).<sup>10</sup> While these results were consistent with the previous report by Odeh and Yamani<sup>46</sup> who mentioned that the peroxide values were acceptable even if they increased to 10 mEq/kg when they studied the chemical quality of Arabic sweets produced in Jordan. Chugh *et al*<sup>47</sup> studied the peroxide value in low fat biscuits and found values lower than 6.5 mEq O<sub>2</sub>/kg of fat after 90 days of storage, these low values can be related to the low fat content of biscuits and the low attitude to oxidize. Kumari *et al*<sup>48</sup> studied the shelf life of biscuits and cookies incorporating germinated pumpkin seed flour and found a peroxide value ranging between 2.81 (after 15 days storage) and 6.33 mEq O<sub>2</sub>/kg of fat after 75 days, confirming the deterioration of biscuits during storage.

#### **Effect of Storage Periods on The Hmf Content in Biscuit Samples Manufactured in Spain, Iran, Uae and Turkey**

HMF is an index of freshness. The lower the HMF the higher the freshness. Figure 5 showed the effect of storage periods on the HMF content in samples from Spanish, Iranian, UAE and Turkish biscuits stored at room temperature for 90 days.





**Fig. 5: Effects of storage periods on HMF contents (mg/kg) in biscuit samples stored at room temperature**

A significant effect ( $p < 0.05$ ) was found for each country of manufacture, types of biscuits and storage, the interaction between the country of manufacture and the types of biscuits, the interaction between the quality of biscuits and storage, and the triple interaction between the country of manufacture and the types of biscuits and storage. The amount of HMF in Spanish biscuit samples were 6.17, 16.30, 7.16, 25.19, 7.41, 38.28, 4.69, 31.86, 49.89 mg/kg for each of BSC<sub>1</sub>-S, BSC<sub>2</sub>-S, BSC<sub>3</sub>-S, BSD<sub>1</sub>-S, BSD<sub>2</sub>-S, BSD<sub>3</sub>-S, BScr<sub>1</sub>-S, BScr<sub>2</sub>-S, and BScr<sub>3</sub>-S respectively when stored at room temperature for 90 days. While the amount of HMF in Iranian biscuit samples were 17.78, 28.65, 111.89, 20.50, 70.88, 73.85, 176.35, 65.70, 149.92 mg/kg for BSC<sub>1</sub>-I, BSC<sub>2</sub>-I, BSC<sub>3</sub>-I, BSD<sub>1</sub>-I, BSD<sub>2</sub>-I, BSD<sub>3</sub>-I, BScr<sub>1</sub>-I, BScr<sub>2</sub>-I, and BScr<sub>3</sub>-I, respectively. For the UAE biscuit samples, the amount of HMF were 8.39, 178.33, 45.44, 104.48, 29.14, 99.29, 41, 74.84 and 49.40 mg/kg for each of BSC<sub>1</sub>-U, BSC<sub>2</sub>-U, BSC<sub>3</sub>-U, BSD<sub>1</sub>-U, BSD<sub>2</sub>-U, BSD<sub>3</sub>-U, BScr<sub>1</sub>-U, BScr<sub>2</sub>-U, and BScr<sub>3</sub>-U, respectively, when stored at room temperature for 90 days. The amounts of HMF in the Turkish biscuit samples were 74.1, 7.9, 10.37, 18.03, 26.92, 9.38, 46.68, 47.67, 143.53 mg/kg for each of BSC<sub>1</sub>-T, BSC<sub>2</sub>-T, BSC<sub>3</sub>-T, BSD<sub>1</sub>-T, BSD<sub>2</sub>-T, BSD<sub>3</sub>-T, BScr<sub>1</sub>-T, BScr<sub>2</sub>-T, and BScr<sub>3</sub>-T, respectively.

The obtained research results agreed with Mankowska *et al.*<sup>49</sup> and Jalili and Ansari<sup>50</sup> who

showed that storage is one of the influencing factors that cause a significant increase in the amount of HMF. Michalak *et al.*<sup>51</sup> also showed that these differences observed in the increase in the amount of HMF in the analyzed samples are due to differences in the components of the products.

#### Effect of Storage Periods on The Microbial Content of Biscuit Samples Manufactured in Spain, Iran, Uae and Turkey

The microbial content is an index of quality of a food in relation to both its manufacturing and storage. Table 5 shows the effects of storage periods on the total count bacteria in samples of Spanish, Iranian, UAE and Turkish biscuits stored for 90 days at room temperature.

A significant effect ( $p < 0.05$ ) was revealed by the statistical analysis for each country of manufacture, types of biscuits and storage, the interaction between the country of manufacture and the types of biscuits, the interaction between the types of biscuits and storage, and the triple interaction between the country of manufacture and the types of biscuits and storage. The total count bacteria ranged from 2.62 – 4.00, 2.80 - 4.20, 2.65 - 3.99, 2.59 - 3.95, 2.87 - 4.27, 2.69 - 4.08, 2.68 - 4.10, 2.66 - 4.12, 2.75 - 4.15 log cfu/g for each of BSC<sub>1</sub>-S, BSC<sub>2</sub>-S, BSC<sub>3</sub>-S, BSD<sub>1</sub>-S, BSD<sub>2</sub>-S, BSD<sub>3</sub>-S, BScr<sub>1</sub>-S, BScr<sub>2</sub>-S, and BScr<sub>3</sub>-S, respectively. While the total count bacteria in the

Iranian biscuit samples ranged from 2.61 - 3.98, 2.50 - 3.92, 2.61 - 4.02, 2.57 - 3.95, 2.63 - 4.02, 2.62 - 4.02, 2.57 - 3.96, 2.59 - 4.00, 2.92 - 5.7) log cfu/g for BSC<sub>1</sub>-I, BSC<sub>2</sub>-I, BSC<sub>3</sub>-I, BSD<sub>1</sub>-I, BSD<sub>2</sub>-I, BSD<sub>3</sub>-I, BScr<sub>1</sub>-I, BScr<sub>2</sub>-I, and BScr<sub>3</sub>-I respectively for a period of 90 days at room temperature. For the total count bacteria in the UAE biscuit samples, it ranged 2.72 - 4.12, 2.51 - 3.91, 2.91 - 4.31, 2.92 - 4.32, 2.71 - 4.11, 3.82 - 5.22, 4.11 - 5.51, 3.90 - 5.30, 2.80 - 4.20 log cfu/g for each of BSC<sub>1</sub>-U, BSC<sub>2</sub>-U, BSC<sub>3</sub>-U, BSD<sub>1</sub>-U, BSD<sub>2</sub>-U, BSD<sub>3</sub>-U, BScr<sub>1</sub>-U, BScr<sub>2</sub>-U, and BScr<sub>3</sub>-U, respectively. The total bacteria count in the Turkish biscuit samples were 2.00 - 3.40, 2.61 - 4.01, 2.82 - 4.22, 4.08 - 5.48, 3.84 - 5.24, 2.83 - 4.23, 2.57 - 3.97, 2.72 - 4.12, 2.63 - 4.03 log cfu/g for each of BSC<sub>1</sub>-T, BSC<sub>2</sub>-T, BSC<sub>3</sub>-T, BSD<sub>1</sub>-T, BSD<sub>2</sub>-T, BSD<sub>3</sub>-T, BScr<sub>1</sub>-T, BScr<sub>2</sub>-T, and BScr<sub>3</sub>-T, respectively. The results showed that 9 of the total 36 types of biscuits examined were less than the microbial limit of 4 log cfu/g as an acceptable limit for the total number

of bacteria in biscuits specified by the Food and Agriculture Organization (FAO/WHO, 2001).<sup>10</sup> These results were consistent with Nagi *et al.*<sup>52</sup> who noted a large variation in the microbial content during the storage period, this significant increase in the microbial load of the baked biscuit samples for a period of three months was due to the significant increase in moisture during the storage period. The results also agreed with Kumari *et al.*<sup>53</sup> and Tlay *et al.*<sup>54</sup> They evaluated the shelf-life of biscuits and cookies by calculating the bacteria total number in the stored samples, as the results showed that the bacteria total number increased significantly with storage period. The data of the microbial content (Table 5) can be compared with the moisture content which facilitates the microbial growth. It can be seen that the moisture content increased with storing and (Fig.4) and microbial content showed the same increasing trend.

**Table 5: Effect of grief periods on the total bacteria count (CFU/mL) for biscuit samples stored at room temperature for 90 days. Values express as mean ± standard deviation (n = 3)**

Biscuit sample	Storage time (days)			
	0	30	60	90
BSC <sub>1</sub> -S	2.62 ± 0.19	2.81 ± 0.17	3.20 ± 0.17	4.00 ± 0.14
BSC <sub>2</sub> -S	2.80 ± 0.17	2.99 ± 0.16	3.39 ± 0.18	4.20 ± 0.16
BSC <sub>3</sub> -S	2.65 ± 0.16	2.86 ± 0.16	3.27 ± 0.17	3.99 ± 0.18 2
BSD <sub>1</sub> -S	2.59 ± 0.16	2.80 ± 0.16	3.39 ± 0.18	3.95 ± 0.14
BSD <sub>2</sub> -S	2.87 ± 0.15	3.06 ± 0.16	3.47 ± 0.18	4.27 ± 0.19
BSD <sub>3</sub> -S	2.69 ± 0.16	2.88 ± 0.16	3.28 ± 0.17	4.08 ± 0.19
BScr <sub>1</sub> -S	2.68 ± 0.14	2.89 ± 0.14	3.30 ± 0.16	4.10 ± 0.19
BScr <sub>2</sub> -S	2.66 ± 0.19	2.88 ± 0.18	3.31 ± 0.16	4.12 ± 0.18
BScr <sub>3</sub> -S	2.75 ± 0.13	2.96 ± 0.14	3.37 ± 0.15	4.15 ± 0.20
BSC <sub>1</sub> -I	2.61 ± 0.14	2.79 ± 0.14	3.18 ± 0.19	3.98 ± 0.05
BSC <sub>2</sub> -I	2.50 ± 0.19	2.71 ± 0.18	3.12 ± 0.17	3.92 ± 0.15
BSC <sub>3</sub> -I	2.61 ± 0.13	2.82 ± 0.19	3.22 ± 0.14	4.02 ± 0.32
BSD <sub>1</sub> -I	2.57 ± 0.19	2.75 ± 0.12	3.15 ± 0.15	3.95 ± 0.16
BSD <sub>2</sub> -I	2.63 ± 0.18	2.82 ± 0.17	3.22 ± 0.14	4.02 ± 0.75
BSD <sub>3</sub> -I	2.62 ± 0.13	2.82 ± 0.16	3.22 ± 0.16	4.02 ± 0.41
BScr <sub>1</sub> -I	2.57 ± 0.14	2.75 ± 0.13	3.16 ± 0.18	3.96 ± 0.20
BScr <sub>2</sub> -I	2.59 ± 0.18	2.78 ± 0.18	3.20 ± 0.20	4.00 ± 0.10
BScr <sub>3</sub> -I	2.92 ± 0.19	3.10 ± 0.18	4.91 ± 0.19	5.71 ± 0.48
BSC <sub>1</sub> -U	2.72 ± 0.15	2.91 ± 0.13	3.32 ± 0.14	4.12 ± 0.05
BSC <sub>2</sub> -U	2.51 ± 0.14	2.72 ± 0.11	3.11 ± 0.01	3.91 ± 0.34
BSC <sub>3</sub> -U	2.91 ± 0.19	3.10 ± 0.21	3.51 ± 0.33	4.31 ± 0.64
BSD <sub>1</sub> -U	2.92 ± 0.18	3.13 ± 0.33	3.52 ± 0.17	4.32 ± 0.03
BSD <sub>2</sub> -U	2.71 ± 0.15	2.93 ± 0.17	3.31 ± 0.24	4.11 ± 0.01



BSD <sub>3</sub> -U	3.82 ± 0.17	4.03 ± 0.10	4.42 ± 0.07	5.22 ± 0.16
BScr <sub>1</sub> -U	4.11 ± 0.15	4.30 ± 1.16	4.71 ± 0.51	5.51 ± 0.14
BScr <sub>2</sub> -U	3.90 ± 0.13	4.10 ± 0.71	4.50 ± 0.10	5.30 ± 0.09
BScr <sub>3</sub> -U	2.80 ± 0.18	3.01 ± 0.33	3.40 ± 0.35	4.20 ± 0.18
BSC <sub>1</sub> -T	2.00 ± 0.17	2.21 ± 0.014	2.60 ± 0.13	3.40 ± 0.08
BSC <sub>2</sub> -T	2.61 ± 0.14	2.83 ± 0.18	3.21 ± 0.18	4.01 ± 0.04
BSC <sub>3</sub> -T	2.82 ± 0.16	3.00 ± 0.20	3.42 ± 0.27	4.22 ± 0.15
BSD <sub>1</sub> -T	4.08 ± 0.18	4.29 ± 0.11	4.68 ± 1.15	5.48 ± 0.31
BSD <sub>2</sub> -T	3.84 ± 0.19	4.04 ± 0.34	4.44 ± 0.09	5.24 ± 0.09
BSD <sub>3</sub> -T	2.83 ± 0.15	3.00 ± 0.27	3.43 ± 0.35	4.23 ± 0.05
BScr <sub>1</sub> -T	2.57 ± 0.14	2.79 ± 0.11	3.17 ± 0.18	3.97 ± 0.15
BScr <sub>2</sub> -T	2.72 ± 0.14	2.90 ± 0.12	3.32 ± 0.27	4.12 ± 0.07
BScr <sub>3</sub> -T	2.63 ± 0.15	2.84 ± 0.11	3.23 ± 0.36	4.03 ± 0.10

### Conclusion

During this study different brands of biscuits were collected from Spanish, Iran, UAE, and Turkey by GC-MS for SFA and TFA and by HPLC for AFB1, AFB2, AFG1, and AFG2) as well as quality properties during storage for 90 days were investigated. It has been observed that the results showed that no AFB2 and AFG2 were found in any biscuits samples regardless of manufacturing country. However, AFB1 was found in BSD<sub>1</sub>-S, BSD<sub>1</sub>-I, BSC<sub>2</sub>-U and BScr<sub>1</sub>-T collected from Spain, Iran, UAE, and Turkey, respectively. The results also showed that G1 was only found BSD<sub>1</sub>-S and BScr<sub>1</sub>-T. The results showed that the values of SFA in cookies, crackers, and digestive for Spanish, Iran, UAE, and Turkey samples ranged from 20.69% to 45.90%, 10.90% to 47.03% and 11.35% to 46.87%, respectively. The results showed that the values of UFA in cookies, crackers, and digestive for Spanish, Iran, UAE, and Turkey samples ranged from 11.31% to 59.17%, 12.40% to 74.24% and ND to 68.79%, respectively. The results showed that the values of TFA in cookies, crackers, and digestive for Spanish, Iran, UAE, and Turkey samples ranged from ND to 21.425%, ND to 25.35% and ND to 4.68%, respectively. A gradual increase in the moisture content and peroxide value during the storage period in all the baked biscuit samples. The results showed that 9 of the total 36 types of biscuits examined were less than the microbial limit of 4 log cfu/g as an acceptable limit

for the total number of bacteria in biscuits specified by the Food and Agriculture Organization. Monitoring biscuit fat is the best strategy to avoid industrially manufactured TFA and excessive SFA. Fortifying biscuits with omega fatty acids might encourage people to eat them and other nutrients. These statistics will help regulate children's and health-conscious consumers' biscuit saturated fat and trans-fat consumption. Knowing aflatoxins' contents helps prevent health issues. The overall qualitative condition of biscuits deteriorated with storage time and suggest to eat these biscuits within 60 days of preparation.

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### Conflicts of Interest

The authors declare no conflict of interest.

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