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Nutritional Quality and Safety Characteristics of Imported Biscuits Marketed in Basrah, Iraq

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Abstract: The ingredients and the preparation methods influence biscuit quality and safety. In Iraq, biscuit imports are increasing every year, but no information is available in the scientific literature on their quality and safety features. This work analyzed three types of biscuits (cookies, crackers, and digestives) sampled in the Basrah markets (Iraq) but produced in Spain, Iran, Turkey, and United Arab Emirates. Nine different brands were considered for each country of origin (n = 36), with three replicates per sample. Moisture, ash, fat, proteins, fiber, water activity, peroxide value, 5-hydroxymethyl-2-furfural (HMF), acrylamide, heavy metals, and microbial load were analyzed. All the nutritional parameters were significantly influenced by the variables "Biscuit type" and "Country". Cookies showed significantly higher fat content and lower protein content than crackers and digestives, as well as higher peroxide value (which was below the limit set by the FAO/WHO within the World Wood Program). Spanish samples had more fat and fewer proteins than biscuits made in other countries. Very high variability was observed in HMF (from not detected to 62.08 mg/kg) and AA content (reaching 1421.8 µg/kg). Cadmium was always absent, and lead was considerably below the allowed limit. Yeasts and molds were above the limits in five samples.

Keywords: bakery product; biscuit; cookie; cracker; digestive; HMF; acrylamide

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1. Introduction

Dough free of biological yeast can be used to make little thin crispy cakes called "biscuits" [1], appreciated for their remarkable shelf life and good eating quality [2]. Biscuits are one of the most popular foods for all ages for their taste, flavor, and low cost [3]. Their long shelf-life helps easy distribution and export [4]. The principal ingredients of biscuits are sugar, fat, water, and flour; while other components include salt, milk, chemical aerating agents, and flouring agents [5].

Microbial pathogens can contaminate foods and cause foodborne diseases [6,7]. The Centers for Disease Control and Prevention (CDCP) in the United States have stated that either foodborne or waterborne pathogens are considered to be the primary causative factors in 76 million cases of illnesses each year in the United States alone [8]. The percentage of pathogenic bacteria, parasites, and viruses was 5 million cases, 2 million cases, and 30 million cases, respectively [9].

A study conducted in Iraq reported the level of microbial (bacteria and fungi) contamination in five types of biscuits from local markets in Baghdad city. The highest number of bacteria was 21.6 × 10^3 cfu/g in Iranian biscuits while the lowest number was 14.3 × 10^3 cfu/g in a local biscuit sample. The highest number of fungi was 16 × 10^3 cfu/g and the lowest number was 5.3 × 10^3 cfu/g in the Iranian and the same local biscuit sample, respectively [10]. The health effects of microbial contamination may be noticed within days

or weeks, while the effects of chemical contamination may take a long time to manifest. Examples of chemical contaminants are heavy metals such as Pb, Cd, Zn, Hg, Mg, Mn, Cu, and Co [4]. Pb and Cd have no known biological functions and may exhibit toxicological problems even at low or trace concentrations [11,12]. Dada et al. [13] mentioned that the highest metal loads were found in biscuit and doughnut samples, hypothesizing the source of contamination firstly during the cultivation of the primary raw material, wheat, and secondly during the processing of the final products.

Thermal processing is widely used in the preparation of food items. The most popular methods are cooking, baking, roasting, extrusion cooking, pasteurization, or sterilization [14]. Several reactions are ongoing during thermal treatments, such as the Maillard reaction, caramelization, and oxidation of lipids [4]. As a consequence, not only compounds with positive sensory impact arise from heating processes but also contaminants, such as acrylamide and 5-hydroxymethyl-2-furfural (HMF) [4]. Cereal products are among the main contributors to acrylamide exposure and therefore are included among the foodstuffs regulated by the European Commission [15]. Recently, acrylamide gained considerable attention due to its toxicity. Additionally, high levels of HMF have demonstrated cytotoxicity, eye inflammation, mucous membranes, and upper breathing tract [16]. In fact, studies in rats and mice have shown that HMF can probably be cancerous [17].

There is currently an increase in the consumption of biscuits as popular snacks, especially in developing countries. However, there is limited knowledge on the nutritional, physic-chemical, and microbiological characteristics of biscuits marketed in these countries, where a poor economy and harsh climatic conditions can make it difficult to comply with hygiene requirements during processing, marketing, and storage. It would be advisable, therefore, to have more data on biscuit quality and safety in developing countries.

In Iraq, biscuit and cake imports have continued to increase every year since 2015. Total imports in these categories accounted for USD 333 million in 2015, USD 513 million in 2016, and USD 525 million in 2017, with the highest share of imports from Turkey [18]. However, except for a single study, which evaluated the microbial contamination of commercial biscuits available in the Iraqi markets [10], no other information is available in the scientific literature on the quality and safety of imported biscuits marketed in Iraq. Therefore, to fill this knowledge gap, the objective of this research was to evaluate the nutritional composition, acrylamide, HMF, heavy metals, and microbiological properties of the commercial biscuits sold in local markets of Basrah, Iraq, imported from other four countries (Spain, Iran, Turkey, and United Arab Emirates). Nine different brands of biscuits per country of origin were collected (n = 36), with three replicates.

2. Materials and Methods

2.1. Sample Collection

Commercial biscuits were purchased in the period from July 2021 to November 2021 from various retail shops and supermarkets in Basrah city, Basrah, Iraq. Three major classes were considered: cookies, crackers, and digestives.

Biscuits were made in Spain, Iran, United Arab Emirates (UAE), and Turkey. Nine different brands of biscuits per country of origin were collected (n = 36), with three replicates. Biscuit coding, origin, and ingredients are reported in Table S1. Sampling plan reflected the most popular brands consumed by different income groups and different age groups, including children. Biscuit samples were finely ground before being analyzed.

2.2. Determination of Moisture

Moisture content was determined by oven-drying at 105 °C for 3 h, then cooling the sample in a desiccator. The process was repeated several times until the weight was constant.

2.3. Determination of Ash

Samples were oven-burned at 600 °C to constant weight and ashes were determined by weighing.

2.4. Fat Determination

The lipid fraction of samples was extracted according to AOAC method 935.38 [19] using petroleum ether (40–60 °C boiling point) as extracting solvent.

2.5. Determination of Crude Protein

The Kjeldahl method was used to determine the crude protein content of the samples by (1) digesting the samples in H_2SO_4 in presence of a catalyst, which results in nitrogen conversion to ammonia; (2) distilling the ammonia in a trapping solution; and (3) quantifying the ammonia by titration with a standard solution. Percent of the crude protein content of the samples was calculated as percent nitrogen × 5.75.

2.6. Crude Fiber Determination

The samples were homogenized and oven-dried to constant weight at 105 °C. To remove the crude fat, samples were extracted with *n*-hexane in Soxhlet units. The dried and defatted samples were heated in 1.25% H_2SO_4 (*v/v*) and then 1.25% NaOH (*w/v*) solutions for 30 min [20]. Finally, in a desiccator furnace, the dried filtrate was fired at 600 °C for 8 h. The following formula was used to compute the percentage of crude fiber:

% Crude fiber = 100× [(Weight of crucible with defatted, dried sample) − (Weight of crucible with ash)]/Weight of the sample.

2.7. Determination of Total Carbohydrate

The available carbohydrate content was calculated by difference according to the following equation:

Carbohydrate content (%) = 100 − [protein + moisture + crude fiber + fat + ash].

2.8. Water Activity

The water activity was determined using relative humidity sensors (Shinyei Technologies, Kobe, Japan). The biscuit samples were dried in an oven at 90 °C. The water activity was determined by dividing the equilibrium relative humidity by 100.

2.9. Peroxide Value

The peroxide value was determined according to AOAC method 965.33 [19]. Five grams of sample were mixed with 30 mL acetic acid–chloroform mixture in a conical flask. Then, 0.5 mL saturated potassium iodide solution was added and titrated against sodium thiosulphate (0.01 N) until the yellow color almost disappeared. After that, 0.05 mL starch solution was added, and the titration was continued to release all iodine from the chloroform layer until the disappearance of the blue color. The blank was performed in the same way. Peroxide value was calculated as meq O_2/kg fat.

2.10. Determination of HMF

The content of hydroxymethyl furfural (HMF) was analyzed according to the protocol described by Švecová and Mojmír [21]. For the extraction of HMF, 3 g of powdered and homogenized sample and 50 mL of deionized water were put into a 100 mL volumetric flask and subjected to an ultrasonic bath for 15 min. After that, 1 mL of Carrez II ($ZnSO_4$ 20%) and 1 mL of Carrez I ($K_4[Fe(CN)_6]$ 15%) were added and completed to 100 mL with deionized water. For analysis, HMF was analyzed by HPLC system (Sykam S 1130, Germany with UV/VIS detector set at 285 nm). Manually, aliquots of samples in the volume of 25 µL were injected. The separation column used was an Arcus Ep-C18 column (4.6 mm × 250 mm, 5 µm). The mobile phase consisted of a mixture of water/acetonitrile in the ratio of 90:10 for preservation of isocratic conditions.

2.11. Determination of Acrylamide

Acrylamide content was determined according to Gökmen et al. [22] with a slight modification. Two grams of sample were mixed with $MgSO_4$ (4 g) and NaCl (1 g) in centrifuge tubes (50 mL). After that, 5 mL of *n*-hexane was added and vortexed for 1 min to help separate the hydrophilic and hydrophobic components of the food. Acetonitrile (10 mL) and distilled water (10 mL) were added and further vortexed for 1 min and later centrifuged (LHW 24958, Wageningen, The Netherlands) at 3000 rpm for 5 min. The resulting aqueous acetonitrile phase (1 mL) was subsequently treated with 1500 and 500 mg of $MgSO_4$ and NaCl, respectively, vortexed and agitated at 4000 rpm for 5 min. Finally, 2 mL of the supernatant was siphoned for HPLC analysis (Sykam S 1130, Eresing, Germany). A Cecil-Adept binary pump HPLC with a Dynamic Absorbance detector was used for the HPLC analysis. The separation column used was an Arcus Ep-C18 column (4.6 mm × 250 mm, 5 µm), and the column oven was set at 40 °C. The mobile phase was made up of acetonitrile and water (20:80 *v/v*). The flow rate of the mobile phase was set at 1 mL/min, and it was detected at 225 nm. A volume of 10 µL was injected into the HPLC for the analysis using a sample injector (S 5300, Sykam, Eresing, Germany). Acrylamide was detected and quantified by matching their peaks with the standard retention time.

2.12. Determination of Heavy Metals and Trace Elements

Atomic absorption spectrometry and flame photometry (Biotech Engineering Management Co., Ltd., Nicosia, Cyprus) were used for the analysis of the content of heavy metals (Pb, Cd) and trace elements (Cu, Zn) according to the protocol described by AOAC method 965.09 [19]. Exactly, 1 g of ground biscuit sample was mixed with 12 mL of HNO_3 in a digesting glass tube and kept overnight at room temperature. The mixture was then treated with 4 mL of $HClO_4$ and kept in the fumes block for digestion. The temperature was gradually raised from 50 °C to 250–300 °C. The mixture was allowed to cool down and the contents of the tubes were transferred to 100 mL volumetric flasks and the volumes were made to 100 mL with distilled water. The wet-digested solution was transferred to clearly labeled plastic bottles. The digest was stored and used for mineral analysis as reported in [23].

2.13. Microbiological Analysis

Aerobic mesophilic bacteria (total count) were determined by the plate count method, on the nutrient agar [24]. *Staphylococcus aureus* was counted using Mannitol salt agar (MSA). In the suspected colonies of *S. aureus*, yellow color referred to Mannitol fermentation, while a yellow halo represented coagulase production around the colony. In addition, catalase, coagulase tests, and Gram staining techniques were performed for suspected colonies for further confirmation. Typical colonies of *S. aureus* were counted to determine cfu per gram of sample [25]. *Escherichia coli* and coliform bacteria were performed on MacConkey agar media by using spread plate method. Plates were inoculated and then incubated at 37 °C for 24 h. The blue-colored colonies were enumerated to calculate total coliform as CFU/g [26]. *Salmonella* was detected in 25 g samples of each seed that had been pre-enriched with 225 mL of tryptic soy broth (TSB; Bioxon, BD) and incubated at 35 °C for 24 h. One mL and 0.1 mL aliquots were transferred to tubes containing 9 mL of tetrathionate broth (Bioxon, BD) and Rappaport Vassiliadis broth (Bioxon, BD), respectively. Differential media (*Salmonella-Shigella* agar) was used to propagate total *Enterobacteriaceae* and *Salmonella-Shigella* numbers in tubes. After 24 h of incubation at 37 °C, the distinctive pale yellow straw colonies to red colonies were enumerated as members of the *Enterobacteriaceae*. Suspect colonies were enumerated in order to calculate the number of CFU per gram of sample [27]. The *Bacillus* spp. bacteria detection was carried out on dextrose–casein–peptone agar. In particular, *Bacillus* spp. was isolated from mannitol–egg yolk–polymyxin (MYP) agar. For each sample, 25 g was homogenized aseptically in 225 mL of sterile Butterfield's phosphate-buffered dilution water. The dilution was heated at 80 °C for 10 min in order to eliminate microbial vegetative forms and 100 mL was spread on MYP

agar and plates were incubated at 37 °C for 24–48 h [28]. Yeasts and molds were determined by using the spread plating method. An amount of 25 g of sample was aseptically weighed and placed in a plastic bag. One gram of the biscuit samples was obtained and transferred into 9 mL of distilled water in a glass beaker, then shaken thoroughly. The serially diluted samples, (0.1 mL) were inoculated over potato dextrose agar and incubated at 25–30 °C for 3–5 days [29].

2.14. Statistical Analysis

The results were statistically analyzed by two-way analysis of variance (ANOVA), at a significance level $\alpha = 0.05$, followed by Tukey's Honestly Significant Differences (HSD) test for post hoc multiple comparisons, in order to estimate the influence of the variables biscuit type and country, and of their first-order interaction, using the software SPSS 13 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Nutritional Composition

Table 1 reports the nutritional composition of different types of biscuits collected in the local markets of Basrah, Iraq. Three categories were considered: cookies, crackers, and digestive biscuits. Crackers, also named "water biscuits", are savory or salty biscuits, whereas the term "cookie" is used for the sweet ones and "digestive" for semisweet biscuits.

The moisture content varied from 1.42 g/100/g to 2.52 g/100 g, without a significant effect by the variables "Biscuit type" and "Country" or by their first-order interaction. The range observed, due to differences in formulation, processing, and packaging conditions, agreed with Pareyt et al. [30], who reported that the moisture content of biscuits varies from 1 to 5 g/100 g. All samples showed a moisture content lower than the allowed maximum limit (4.5 g/100 g) established by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) [31].

The ash content ranged from 0.48 g/100 g to 3.19 g/100 g and was significantly influenced by the variables "Biscuit type" ($p < 0.001$) and "Country" ($p < 0.01$). The effect of the "Biscuit type*Country" interaction, instead, was not significant. The highest ash contents were observed in the cracker category, and in Turkish samples. Ash content can be affected by the number of preservatives and leavening agents added [32]. In addition, high levels of ash are generally associated with the addition of bran [33]. The content of ash found in all biscuit samples was lower than the limit of 3.5 g/100 g established by the FAO/WHO [31].

Although not perceived as fatty foods by consumers, biscuits usually contain relevant amounts of fats. However, contrarily to the current trend established in Western countries, where research efforts are made to lower the fat content of biscuits and improve their quality [34–36], in developing countries the perception of fat is not negative because children's malnutrition still exists, and hi-energy food products are required. Therefore, the FAO/WHO, within the World Food Program (WFP), sets a minimum limit for the fat content of biscuits, accounting for 15 g/100 g [31]. This limit was met by the majority of the examined biscuits, with only five samples much below 15 g/100 g, having fat contents in the range 6.83–11.81 g/100 g. Four of these samples were Turkish and one was Iranian. Rutkowska et al. [37], instead, found fat content values lower than 15 g/100 g in nine out of twelve biscuits representative of four Polish confectionery producers, as a consequence of the request for decreasing fat content of food generally expressed by the European consumers.

The fat content of the examined biscuits varied in a broad range, from 6.52 g/100 g to 26.86 g/100 g, and was significantly affected by the variables "Biscuit type" ($p < 0.001$) and "Country" ($p < 0.05$), but was not significantly influenced by their first-order interaction. The highest fat contents were observed in cookies, and in Spanish samples.

Table 1. Nutritional composition of biscuits marketed in Basrah, Iraq.

Biscuit Type	Moisture (g/100 g)	Ash (g/100 g)	Fiber (g/100 g)	Fat (g/100 g)			Protein (g/100 g)			Carbohydrates (g/100 g)
				D	L	Δ%	D	L	Δ%	
Cookies										
BSC1-S	1.96 ± 0.10	1.23 ± 0.05	0.41 ± 0.03	22.41 ± 0.26	24	−6.6	7.63 ± 0.09	5.5	38.7	66.36 ± 2.02
BSC2-S	2.17 ± 0.05	1.19 ± 0.03	0.37 ± 0.01	23.77 ± 0.57	24	−0.9	6.14 ± 0.04	5.9	4.1	66.39 ± 3.17
BSC3-S	2.07 ± 0.08	1.05 ± 0.07	0.26 ± 0.02	26.86 ± 0.32	27	0.5	4.93 ± 0.15	4.4	12.2	64.83 ± 4.09
BSC1-I	1.85 ± 0.05	0.48 ± 0.11	0.25 ± 0.01	21.50 ± 0.51	NR	-	6.72 ± 0.21	NR	-	69.40 ± 4.02
BSC2-I	1.42 ± 0.07	1.37 ± 0.13	0.45 ± 0.04	23.88 ± 0.59	25.3	−5.6	8.95 ± 0.13	5.2	72.1	63.93 ± 3.71
BSC3-I	2.12 ± 0.11	0.66 ± 0.09	0.14 ± 0.03	14.63 ± 0.16	5.1	187	10.66 ± 0.14	2.2	367	71.85 ± 4.05
BSC1-U	2.13 ± 0.15	0.57 ± 0.13	0.11 ± 0.01	25.77 ± 0.31	25.2	2.2	8.10 ± 0.05	4.9	65.3	63.34 ± 3.11
BSC2-U	1.70 ± 0.11	1.16 ± 0.08	0.31 ± 0.05	16.20 ± 0.14	19.3	16.1	8.93 ± 0.08	6.5	37.4	71.71 ± 4.31
BSC3-U	2.24 ± 0.06	0.92 ± 0.05	0.15 ± 0.04	19.79 ± 0.61	24.3	8.5	9.26 ± 0.05	5.0	85.2	67.64 ± 3.41
BSC1-T	1.50 ± 0.09	1.43 ± 0.26	0.62 ± 0.07	11.10 ± 0.12	10.3	7.7	9.72 ± 0.06	5.4	80.0	75.65 ± 4.41
BSC2-T	1.93 ± 0.12	1.23 ± 0.08	0.42 ± 0.05	24.14 ± 0.16	24	0.5	10.46 ± 0.16	6.1	74.3	61.84 ± 3.21
BSC3-T	2.18 ± 0.19	1.21 ± 0.05	0.45 ± 0.07	26.19 ± 0.17	26	0.7	8.90 ± 0.05	5.0	78.0	61.12 ± 3.17
Crackers										
BSCr1-S	2.05 ± 0.09	2.36 ± 0.07	0.82 ± 0.14	18.44 ± 0.74	20	−7.8	10.96 ± 0.27	6.0	82.6	65.39 ± 3.21
BSCr2-S	2.06 ± 0.06	1.44 ± 0.07	0.59 ± 0.06	21.95 ± 0.12	21	4.5	10.01 ± 0.18	9.5	5.3	63.95 ± 3.41
BSCr3-S	2.14 ± 0.06	1.15 ± 0.06	0.24 ± 0.04	14.53 ± 0.32	14	3.7	2.06 ± 0.14	1.7	21.2	79.88 ± 4.51
BSCr1-I	2.03 ± 0.06	2.50 ± 0.14	0.81 ± 0.12	6.83 ± 0.32	6	13.8	15.07 ± 0.16	9.2	63.8	72.77 ± 4.81
BSCr2-I	1.81 ± 0.06	2.02 ± 0.19	0.65 ± 0.09	19.80 ± 0.58	20.8	−4.8	8.50 ± 0.11	8.5	0	67.22 ± 3.11
BSCr3-I	2.23 ± 0.13	1.85 ± 0.11	0.67 ± 0.10	14.22 ± 0.09	NR	-	12.15 ± 0.07	NR	-	68.95 ± 3.90
BSCr1-U	2.52 ± 0.11	2.37 ± 0.21	0.86 ± 0.13	18.05 ± 0.12	2.5	622	13.54 ± 0.13	2.1	577	62.72 ± 3.11
BSCr2-U	2.33 ± 0.09	1.31 ± 0.11	0.42 ± 0.08	19.42 ± 0.07	NR	-	9.48 ± 0.03	NR	-	67.06 ± 3.31
BSCr3-U	2.18 ± 0.11	1.63 ± 0.09	0.51 ± 0.06	21.16 ± 0.07	NR	-	10.05 ± 0.06	NR	-	64.46 ± 3.69
BSCr1-T	1.52 ± 0.08	2.75 ± 0.09	0.92 ± 0.07	11.81 ± 0.14	5	136	11.33 ± 0.58	2.1	493	71.69 ± 4.31
BSCr2-T	1.85 ± 0.07	3.19 ± 0.29	1.51 ± 0.13	9.52 ±0.11	9.5	0.5	10.64 ± 0.57	9.4	13.1	73.29 ± 4.05
BSCr3-T	1.95 ± 0.05	2.92 ± 0.08	1.13 ± 0.13	6.52 ± 0.09	9.5	13.8	12.15 ± 0.07	9.4	29.1	75.36 ± 4.25
Digestives										
BSD1-S	2.04 ± 0.08	1.51 ± 0.06	0.63 ± 0.09	19.21 ± 1.02	21	−8.5	11.09 ± 1.02	8.1	36.9	65.52 ± 3.90
BSD2-S	2.18 ± 0.11	1.14 ± 0.05	0.23 ± 0.06	22.17 ± 0.09	23	5.7	7.94 ± 0.14	7.6	4.4	66.34 ± 3.16
BSD3-S	2.06 ± 0.17	1.24 ± 0.13	0.39 ± 0.05	15.72 ± 0.44	14	2.2	7.03 ± 0.11	6.5	8.1	73.56 ± 4.08
BSD1-I	1.63 ± 0.08	1.27 ± 0.07	0.41 ± 0.06	16.84 ± 0.27	10	68.4	9.29 ± 0.05	4.1	132	70.57 ± 4.21
BSD2-I	1.98 ± 0.11	2.05 ± 0.14	0.66 ± 0.08	17.76 ± 0.25	17	4.4	12.22 ± 0.14	9.1	35.7	65.33 ± 3.61
BSD3-I	1.90 ± 0.03	1.33 ± 0.08	0.42 ± 0.08	14.74 ± 0.18	16	92.1	9.64 ± 0.12	NR	-	71.97 ± 3.52
BSD1-U	2.23 ± 0.11	1.50 ± 0.05	0.64 ± 0.10	18.67 ± 0.12	8.5	219	10.20 ± 0.26	3.0	240	66.76 ± 3.27
BSD2-U	1.80 ± 0.09	1.70 ± 0.12	0.73 ± 0.12	21.33 ± 0.08	24.0	11.1	12.96 ± 0.11	9.2	40.8	61.51 ± 3.11
BSD3-U	2.31 ± 0.12	1.44 ± 0.07	0.64 ± 0.09	20.91 ± 0.18	21.4	2.2	8.11 ± 0.05	7.8	3.9	66.64 ± 4.31
BSD1-T	2.41 ± 0.09	1.74 ± 0.06	0.75 ± 0.12	17.14 ± 0.08	16.9	1.4	7.28 ± 0.04	6.2	17.4	70.74 ± 4.31
BSD2-T	2.28 ± 0.11	1.78 ± 0.11	0.71 ± 0.09	14.75 ± 0.06	8.6	1.5	9.33 ± 0.12	2.8	233	71.15 ± 4.19
BSD3-T	2.21 ± 0.07	1.73 ± 0.12	0.76 ± 0.08	16.81 ± 0.08	16.9	−0.5	11.15 ± 0.17	6.2	79.8	67.40 ± 3.41
Mean cookies	1.93 ± 0.18	0.95 ± 0.08	0.32 ± 0.04	21.35 ± 1.24	-	-	8.36 ± 0.38	-	-	67.01 ± 3.13
Mean crackers	2.05 ± 0.11	2.12 ± 0.11	0.76 ± 0.09	15.18 ± 1.05	-	-	10.49 ± 0.42	-	-	68.97 ± 3.49
Mean digestive	2.08 ± 0.14	1.53 ± 0.09	0.58 ± 0.08	17.99 ± 1.21	-	-	9.68 ± 0.56	-	-	68.12 ± 3.66
Mean Spain	2.08 ± 0.13	1.37 ± 0.07	0.43 ± 0.03	20.56 ± 1.13	-	-	7.53 ± 0.37	-	-	68.01 ± 3.08
Mean Iran	1.88 ± 0.16	1.51 ± 0.08	0.45 ± 0.04	16.69 ± 1.08	-	-	10.35 ± 0.51	-	-	69.09 ± 3.11
Mean UAE	2.16 ± 0.11	1.42 ± 0.09	0.46 ± 0.05	20.14 ± 1.21	-	-	10.07 ± 0.21	-	-	67.64 ± 3.24
Mean Turkey	1.96 ± 0.15	1.99 ± 0.07	0.77 ± 0.02	15.33 ± 1.16	-	-	10.01 ± 0.34	-	-	69.94 ± 3.01
WFP limit	<4.5	<3.5	<2.3	>15	-	-	>10	-	-	-
Significance of variables and interactions										
"Biscuit type"	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$	-	-	$p < 0.01$	-	-	NS
"Country"	NS	$p < 0.01$	$p < 0.001$	$p < 0.05$	-	-	$p < 0.01$	-	-	NS
"Biscuit type*Country"	NS	NS	NS	NS	-	-	NS	-	-	NS

BSC: cookie; BSD: digestive; BSCr: cracker; S = made in Spain; I = made in Iran; U = made in the United Arab Emirates; T = made in Turkey; WFP = FAO/WHO World Wood Program [31]; NR = not reported; D: determined; L: labeled; Δ%: percent difference, calculated as follows: [(Analytically determined value—Value reported in the label/Value reported in the label) × 100]; NS= Not significant. Values are expressed as mean ± standard deviation ($n = 3$).

Fats have an important lubricating function in biscuit-making and positively influence the sensory features of the final product. The higher fat content observed in cookies, compared to the other two categories considered, namely crackers and digestives, was due to their different preparation procedure. Crackers and digestives, indeed, contrarily to cookies, are generally made by layering dough, which requires a good protein network and lower fat content, while cookies arise from an incoherent dough shaped by pressure

into the cavities of the rotative biscuit-making machine, without needing a high protein content and tolerating high-fat levels.

Table 1 reports also the fat content reported on the labels of biscuit packages. Interestingly, in almost all the cases the experimental findings and the fat content values reported in the respective labels were different. In the majority of cases (25 cases out of 36) the reported fat content was lower than the determined one. The magnitude of this difference was relatively limited in the samples manufactured in Spain (from −6.6% to 12.2%), but was really high in some samples from UAE and Iran, reaching a 622% difference in one case (namely BSD1-U, from UAE). This large difference might be due to incorrect use of information in the food composition databases used to calculate the nutritional facts (when the labeled values were calculated, instead of being analytically determined), or might be due to different methods of analysis. Moreover, the label of two Iranian and two UAE biscuit samples did not report the nutritional facts.

Proteins ranged from 2.06 g/100 g to 13.54 g/100 g. The content of proteins was significantly affected by the variables "Biscuit type" ($p < 0.01$) and "Country" ($p < 0.01$), but was not significantly influenced by their first-order interaction. Crackers and digestive samples showed higher protein contents than cookies due to the different preparation procedures, with higher proteins needed to easily obtain a dough sheet. Furthermore, Spanish biscuits showed the lowest protein content. The observed range roughly agreed with Passos et al. [38] who found levels of protein from 3 g/100 g to 14.6 g/100 g for a cream cracker. Rodrigues et al. [39] reported that the level of protein varied from 5.7 g/100 g to 10 g/100 g among four types of industrial biscuits. However, the number of proteins found in the various biscuit types complied in only 16 cases out of 36 with the minimum limit of 10 g/100 g set by the FAO/WHO [31].

Additionally, the labeled protein content, with the only exception of BSCr2-I, differed from the values analytically determined in the lab. The magnitude of the difference was approximatively similar to the one observed for fats, with the highest value accounting for 577% (observed in the same sample, namely BSD1-U, from UAE, which exhibited also the highest difference between labeled and reported fat content).

The content of fiber, ranging from 0.11 g/100 g to 1.51 g/100 g was significantly affected by the variables "Biscuit type" ($p < 0.001$) and "Country" ($p < 0.001$). The effect of the "Biscuit type*Country" interaction, instead, was not significant. The highest fiber content was observed in crackers, and in the Turkish samples. From a Western countries' perspective, the observed values may be considered very low, but it has to be considered that the FAO/WHO, within the World Food Program [31], established, instead, a maximum limit for fiber content accounting for 3.5 g/100 g, due to the need to provide readily bioavailable nutrients more than fiber to face malnutrition. In this view, the content of fiber found in all biscuit types was acceptable, because it was lower than the FAO/WHO limit. However, in view of a generally improved nutrition state of the population in the developing countries, an increase in the fiber content would be advisable, by adding whole meal flours as an ingredient in the biscuits. High fiber intake specifically contributes to reducing appetite, improving glycemic control and insulin sensitivity, and beneficially modulating the composition and activity of intestinal microbiota [40]. Despite the importance of this information, most of the examined biscuit labels did not disclose the fiber content.

The total carbohydrate content (TCC) of commercial biscuits ranged from 61.12 g/100 g to 79.88 g/100 g. Variations in carbohydrate contents, which were calculated by difference, were the consequence of the variations observed in the other nutrients (fats, proteins, fiber). No significant effect on the total carbohydrate content was observed by the variables "Biscuit type" and "Country" or by their first-order interaction.

3.2. Water Activity and Peroxide Value

The water activity of the evaluated biscuits was in the range of 0.21–0.49, without a significant effect by the variables "Biscuit type" and "Country" or by their first-order interaction (Table 2).

Table 2. Water activity and peroxide value of biscuits marketed in Basrah, Iraq.

Biscuit Type	Water Activity	Peroxide Value (meq O_2/kg Fat)
Cookies		
BSC1-S	0.29 ± 0.02	1.66 ± 0.08
BSC2-S	0.38 ± 0.04	1.75 ± 0.09
BSC3-S	0.35 ± 0.04	2.25 ± 0.11
BSC1-I	0.28 ± 0.01	1.41 ± 0.08
BSC2-I	0.21 ± 0.02	1.81 ± 0.07
BSC3-I	0.31 ± 0.02	1.55 ± 0.05
BSC1-U	0.36 ± 0.02	2.05 ± 0.12
BSC2-U	0.27 ± 0.01	1.57 ± 0.11
BSC3-U	0.36 ± 0.02	1.11 ± 0.09
BSC1-T	0.22 ± 0.01	1.45 ± 0.08
BSC2-T	0.29 ± 0.02	1.91 ± 0.11
BSC3-T	0.39 ± 0.04	2.15 ± 0.09
Crackers		
BSCr1-S	0.33 ± 0.01	0.92 ± 0.08
BSCr2-S	0.34 ± 0.04	1.53 ± 0.04
BSCr3-S	0.37 ± 0.01	1.59 ± 0.05
BSCr1-I	0.32 ± 0.04	1.25 ± 0.06
BSCr2-I	0.27 ± 0.01	1.55 ± 0.09
BSCr3-I	0.41 ± 0.04	1.78 ± 0.09
BSCr1-U	0.29 ± 0.04	0.96 ± 0.04
BSCr2-U	0.42 ± 0.02	1.04 ± 0.09
BSCr3-U	0.39 ± 0.02	1.32 ± 0.02
BSCr1-T	0.21 ± 0.01	0.46 ± 0.03
BSCr2-T	0.28 ± 0.02	0.31 ± 0.05
BSCr3-T	0.31 ± 0.04	0.23 ± 0.09
Digestives		
BSD1-S	0.32 ± 0.03	1.11 ± 0.05
BSD2-S	0.39 ± 0.02	1.61 ± 0.11
BSD3-S	0.34 ± 0.01	1.69 ± 0.09
BSD1-I	0.26 ± 0.03	1.79 ± 0.11
BSD2-I	0.29 ± 0.01	0.83 ± 0.04
BSD3-I	0.28 ± 0.02	1.57 ± 0.12
BSD1-U	0.39 ± 0.03	0.94 ± 0.09
BSD2-U	0.27 ± 0.02	1.35 ± 0.08
BSD3-U	0.42 ± 0.02	1.21 ± 0.13
BSD1-T	0.48 ± 0.04	0.81 ± 0.09
BSD2-T	0.41 ± 0.03	0.57 ± 0.06
BSD3-T	0.39 ± 0.03	0.78 ± 0.03
Mean cookies	0.38 ± 0.11	1.72 ± 0.06
Mean crackers	0.32 ± 0.09	1.07 ± 0.03
Mean digestive	0.35 ± 0.08	1.18 ± 0.04
Mean Spain	0.34 ± 0.07	1.56 ± 0.09
Mean Iran	0.29 ± 0.05	0.88 ± 0.09
Mean UAE	0.35 ± 0.05	1.04 ± 0.08
Mean Turkey	0.33 ± 0.06	1.28 ± 0.09
WFP limit	-	<10
	Significance of variables and interactions	
"Biscuit type"	NS	$p < 0.05$
"Country"	NS	NS
"Biscuit type*Country"	NS	NS

BSC: cookie; BSD: digestive; BSCr: cracker; S = made in Spain; I = made in Iran; U = made in the United Arab Emirates; T = made in Turkey; WFP = FAO/WHO World Wood Program [31]; ND = not detected; NS: Not significant. Values are expressed as mean ± standard deviation (n = 3).

It is well understood that an increase in salt, sugar, and reducing chemicals correlates to a decrease in water activity [41]. This finding indicates that the water activity of the majority of biscuits was within the critical a_w limit of 0.43 for biscuits and crackers [42] (considered a critical factor for microbial growth as microorganisms cease their growth in

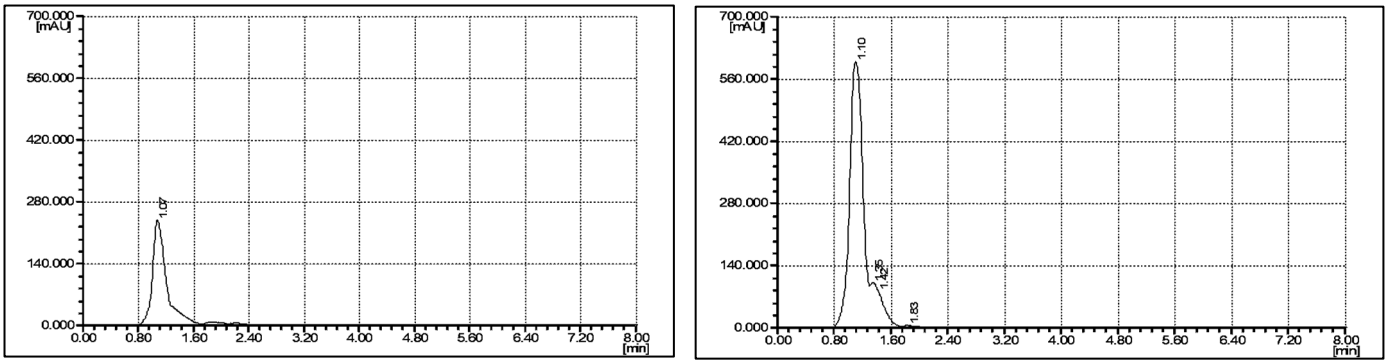
environments with a water activity below 0.60), and lower than 0.63 reported by Schmidt and Fontana [43]. The water activity of the analyzed biscuits ranged between 0.21–0.5, in agreement with Chowdhury et al. [44] who reported that biscuits usually had a water activity of about 0.3. However, our results were not in agreement with Valková et al. [45] who found the water activity of the biscuits ranged from 0.46 to 0.85 after two hours of biscuits baking.

The peroxide value is a critical indicator for the development of primary oxidation of lipids. The peroxide value of the samples ranged from 0.23 to 2.25 meq O_2/kg fat (Table 2) and was significantly influenced ($p < 0.05$) by the "Biscuit type" variable, while the "Country" variable and the "Biscuit type*Country" interaction did not exert a significant effect. The highest peroxide values were observed in cookies, which were the category with the highest fat content. Light, oxygen, and water activity have an impact on the rate of fat oxidation. Nwosu and Akubor [46] confirmed that low ambient temperature delayed the development of peroxides in biscuits during the early months of storage. High temperatures, instead, are known to hasten the pace of oxidative rancidity and the generation of peroxides.

The permeability of packaging materials to light and oxygen varied from one sample to another. However, overall, the peroxide values observed in the different biscuits were low and always considerably below the maximum limit of 10 meq O_2/kg fat set by the FAO/WHO [31].

3.3. HMF and AA Content of Biscuits Samples

The collected biscuits were also examined for their content of HMF and AA. The HPLC resolution for HMF was good (Figure 1a) and peaks were very clear, with the highest level of HMF found in the BSC2-U sample (Figure 1b).



(**a**) (**b**)

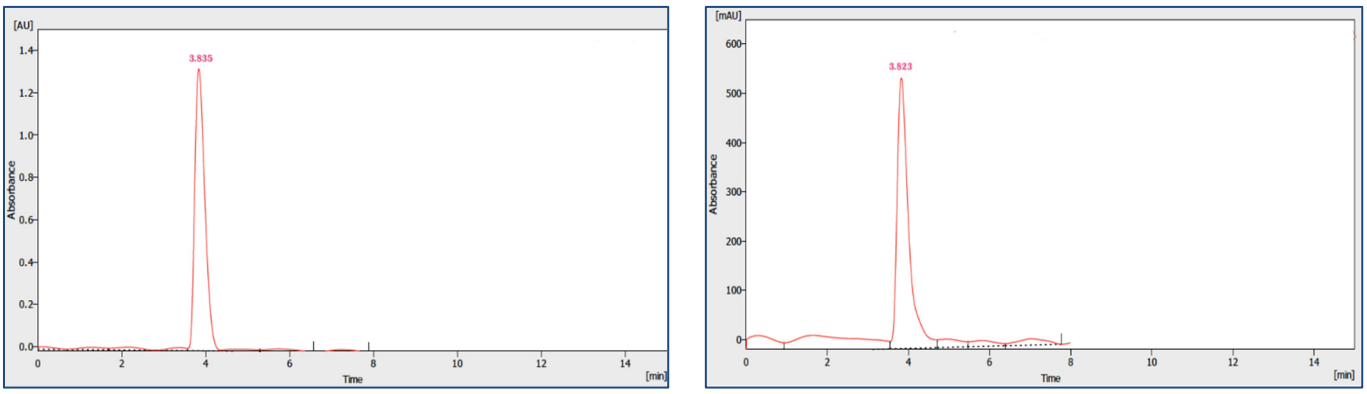
Figure 1. (**a**) HPLC chromatogram of HMF standard; (**b**) HPLC chromatogram of HMF content of the biscuit sample (BSC2-U) which showed the highest level of HMF.

Figure 2a shows the HPLC chromatogram for the AA standard and Figure 2b shows the sample with the highest level of AA, i.e., BSC2-U. Again, the HPLC resolution was good and the peaks were very neat.

Table 3 shows the content of HMF, and AA ascertained in the examined biscuit samples. Extremely high variability in HMF content was observed among samples within the same category or country, from ND to 62.08 mg/kg, which made not significant the influence of the "Biscuit type" and "Country" variables and made not significant also the effect of the "Biscuit type*Country" interaction.

The observed variation might be due to the type of sweetener used in the biscuit recipe. Invert sugar syrup was utilized as a sweetener in certain samples, as opposed to sucrose, which was used in other biscuits. Nguyen et al. [47] demonstrated that HMF rises more rapidly from glucose and fructose than it does from sucrose, which may account for the observed differences. Ameur et al. [48] also observed that the generation of HMF

is temperature-dependent (200 °C, 250 °C, and 300 °C) and sugar-dependent (fructose, glucose, and sucrose). At a higher baking temperature, the amount of HMF was greater: biscuits prepared at temperatures >300 °C contained between 10 and 100 times the amount of HMF (167.4 and 1100.1 mg/kg) as those baked at 200 °C (9.9 to 39.6 mg/kg) [48].



(**a**) (**b**)

Figure 2. (**a**) HPLC chromatogram of acrylamide standard. (**b**) HPLC chromatogram of acrylamide of a biscuit sample (BSC2-U).

Table 3. Content (µg/kg) of 5-hydroxymethyl-2-furfural (HMF) and acrylamide (AA) ascertained in biscuits marketed in Basrah, Iraq.

Biscuit Type	HMF (mg/kg)	AA (µg/kg)
Cookies		
BSC1-S	ND	ND
BSC2-S	1.85 ± 0.05	ND
BSC3-S	ND	ND
BSC1-I	3.31 ± 0.15	ND
BSC2-I	5.64 ± 0.12	ND
BSC3-I	32.62 ± 1.50	639.9 ± 4.1
BSC1-U	ND	ND
BSC2-U	62.08 ± 3.11	1421.7 ± 5.7
BSC3-U	9.27 ± 1.03	ND
BSC1-T	19.03 ± 1.14	397.9 ± 3.1
BSC2-T	ND	ND
BSC3-T	ND	ND
Crackers		
BSCr1-S	0.60 ± 0.08	ND
BSCr2-S	6.30 ± 0.74	ND
BSCr3-S	13.55 ± 0.87	243.4 ± 2.9
BSCr1-I	60.66 ± 2.81	1061.9 ± 6.1
BSCr2-I	16.95 ± 1.04	254.3 ± 2.7
BSCr3-I	56.52 ± 2.86	802.4 ± 4.3
BSCr1-U	8.77 ± 1.01	ND
BSCr2-U	19.55 ± 1.24	404.2 ± 3.0
BSCr3-U	13.65 ± 1.04	312.9 ± 3.2
BSCr1-T	9.73 ± 0.75	327.2 ± 3.8
BSCr2-T	10.66 ± 1.11	323.2 ± 3.1
BSCr3-T	54.80 ± 3.11	742.7 ± 4.2
Digestives		
BSD1-S	5.45 ± 0.06	ND
BSD2-S	ND	ND
BSD3-S	7.53 ± 0.62	226.2 ± 3.1

Table 3. *Cont.*

Biscuit Type	HMF (mg/kg)	AA (μg/kg)
BSD1-I	4.68 ± 0.08	ND
BSD2-I	17.14 ± 1.08	273.6 ± 2.6
BSD3-I	17.31 ± 1.09	315.7 ± 3.1
BSD1-U	32.10 ± 2.21	604.7 ± 3.6
BSD2-U	5.64 ± 0.94	ND
BSD3-U	30.81 ± 2.11	597.8 ± 4.0
BSD1-T	3.96 ± 0.94	ND
BSD2-T	6.37 ± 0.86	ND
BSD3-T	ND	ND
Mean cookies	11.15 ± 16.09	202.9 ± 341.8
Mean crackers	22.64 ± 10.45	372.3 ± 257.1
Mean digestive	10.91 ± 8.08	167.9 ± 216.1
Mean Spain	3.92 ± 7.14	52.1 ± 103.6
Mean Iran	23.87 ± 20.52	371.6 ± 107.9
Mean UAE	20.21 ± 26.37	370.2 ± 287.3
Mean Turkey	11.61 ± 25.31	198.7 ± 245.4
EU Limit (Reg. 2017/2158)	-	<350
Significance of variables and interactions		
"Biscuit type"	NS	NS
"Country"	NS	NS
"Biscuit type*Country"	NS	NS

BSC: cookie; BSD: digestive; BSCr: cracker; S = made in Spain; I = made in Iran; U = made in the United Arab Emirates; T = made in Turkey; ND = not detected; NS: Not significant. Values are expressed as mean ± standard deviation (n = 3).

Additionally, the ingredients have an effect on HMF formation: sucrose-containing biscuits had the lowest HMF content (9.9 mg/kg) at 200 °C than biscuits whose formulation included fructose and glucose (39.6–34.2 mg/kg), owing to sucrose's thermal breakdown [48]. Another factor able to dramatically increase the HMF content is the use of ammonium bicarbonate [49], which was present as a leavening agent in the formulation of many of the examined samples (Supplementary Table S1). However, Delgado Andrade et al. [50] stated that the variations in HMF are more related to the type of thermal treatment used in the manufacturing process than to any specific ingredient.

Overall, the HMF content determined in the examined biscuits was consistent with the findings of Ait-Ameur et al. [51], who found HMF ranging from 0.5 mg/kg to 78.6 mg/kg in biscuits marketed in France, and Petisca et al. [52], who ascertained amounts of HMF ranging from 1.65 mg/kg to 82.78 mg/kg.

Results for AA paralleled those of HMF being both AA and HMF contaminants of thermal origin [4]. Extremely high variability was observed also for the AA content among samples within the same category or country. This variability ranged from ND to 1421.7 μg/kg, making not significant the influence of the "Biscuit type" and "Country" variables, as well as the effect of the "Biscuit type*Country" interaction. Differences in raw material composition, such as free asparagine and reducing sugar content, food product compositions, processing techniques, and parameters such as water content, high temperature (more than 120 °C), and time could be the sources of variation in AA levels, as reported in other studies [4,53]. Gökmen et al. [22] discovered that the AA concentration of biscuits containing dietary fiber was greater than that of regular biscuits (261–486 μg/kg) and for crackers values ranged from (30–582 μg/kg) [54]. This might be explained by the presence of asparagine, the primary precursor of acrylamide in cereal products (concentrated in the bran, particularly in wheat bran). Corn and rice, on the other hand, are known to be grains with low asparagine content, which supports a low AA generation in these biscuits [55]. Furthermore, Rufian-Henares et al. [56] demonstrated that the concentration of AA in biscuits produced with different leavening agents, namely ammonium bicarbonate or sodium bicarbonate, differed. Another source of variation might be the type of sugar utilized, which have an important influence on the synthesis and concentration of acrylamide. According

to Ramadan [57], substituting inverted sugar with sucrose in wheat crackers lowered the AA content by 60%.

Our findings were comparable to those of a Swedish study of the AA content in cookies/biscuits/wafers (300 and 230 µg/kg, respectively), and a prospective study on Italian biscuits (200–298 µg/kg) [58,59]. The results were in the same order of magnitude as those reported by Matthys et al. [60] for traditional Belgian biscuits (range 20–1514 µg/kg) and, more recently, by Alyousef et al. [61] for Syrian corn/wheat-based biscuits (range 57–1433 µg/kg). However, certain samples exceeded the mean value of 495 µg/kg (n = 27) found by Cengiz and Gündüz [62] during the assessment of AA exposure among Turkish toddlers from various cereal-based products.

The European Regulation declared 350 µg/kg as the AA limit in cookies and wafers [63]. This limit was met by all the samples made in Spain but was not met by many of the samples made outside the EU. Though these findings did not have legal implications, as Turkey, Iran, and UAE are not subjected to the EU rules, health-related considerations suggest taking action for lowering the AA content. The variations in AA contents between the same item of different brands are worth investigating because they may provide clues on how to produce the same product with a lower AA content if collaborative studies between the industry and research bodies could be established and the differences in raw material properties, processing regimes, and the ingredients used in these products could be obtained clearly from the processors. This type of information may also help consumers in choosing brands with lower AA levels. As a result, the industry may be pushed to reduce AA levels in baked goods.

3.4. Heavy Metals of Biscuits Samples

Table 4 shows the content of trace elements (Zn, Cu) and heavy metals (Pb, Cd) found in the examined biscuits.

Table 4. Trace elements (Zn, Cu) and heavy metal (Pb, Cd) content of biscuits marketed in Basrah, Iraq.

Biscuit Type	Zn (mg/kg)	Cu (mg/kg)	Pb (µg/kg)	Cd (µg/kg)
Cookies				
BSC1-S	1.46 ± 0.44	5.16 ± 0.31	2.07 ± 0.06	ND
BSC2-S	4.03 ± 0.17	6.41 ± 0.31	2.12 ± 0.14	ND
BSC3-S	6.76 ± 0.55	5.43 ± 0.19	3.29 ± 0.21	ND
BSC1-I	6.17 ± 0.43	2.73 ± 1.06	10.92 ± 0.38	ND
BSC2-I	11.87 ± 0.36	2.80 ± 0.18	9.67 ± 0.62	ND
BSC3-I	10.61 ± 0.44	1.99 ± 0.75	14.27 ± 0.91	ND
BSC1-U	2.52 ± 0.38	2.15 ± 0.45	8.94 ± 1.27	ND
BSC2-U	4.55 ± 0.33	4.97 ± 0.11	2.74 ± 0.51	ND
BSC3-U	8.68 ± 0.24	2.06 ± 0.29	14.12 ± 1.29	ND
BSC1-T	2.51 ± 0.62	2.96 ± 0.41	12.08 ± 0.21	ND
BSC2-T	5.15 ± 0.12	5.13 ± 0.18	8.43 ± 0.14	ND
BSC3-T	9.22 ± 0.77	4.31 ± 0.11	8.66 ± 0.11	ND
Crackers				
BSCr1-S	9.12 ± 0.06	3.47 ± 0.17	3.18 ± 0.08	ND
BSCr2-S	4.01 ± 0.23	4.61 ± 0.19	3.51 ± 0.13	ND
BSCr3-S	10.45 ± 0.32	4.01 ± 0.54	3.97 ± 0.09	ND
BSCr1-I	10.75 ± 0.38	1.82 ± 0.51	29.97 ± 0.16	ND
BSCr2-I	5.35 ± 0.52	2.67 ± 0.41	14.34 ± 0.82	ND
BSCr3-I	13.12 ± 0.78	5.49 ± 0.21	8.98 ± 0.47	ND
BSCr1-U	10.23 ± 0.25	1.41 ± 0.19	2.77 ± 0.41	ND
BSCr2-U	5.14 ± 0.15	4.59 ± 0.21	4.99 ± 0.16	ND
BSCr3-U	11.54 ± 0.24	5.51 ± 0.22	6.62 ± 0.43	ND
BSCr1-T	10.87 ± 0.23	4.41 ± 0.31	20.98 ± 0.13	ND
BSCr2-T	6.66 ± 0.31	3.13 ± 0.14	15.01 ± 0.12	ND
BSCr3-T	12.45 ± 0.46	4.68 ± 0.14	20.61 ± 7.79	ND

Table 4. *Cont.*

Biscuit Type	Zn (mg/kg)	Cu (mg/kg)	Pb (μg/kg)	Cd (μg/kg)
Digestives				
BSD1-S	11.06 ± 0.27	4.02 ± 0.79	3.41 ± 0.07	ND
BSD2-S	6.81 ± 0.33	4.45 ± 0.29	3.46 ± 0.66	ND
BSD3-S	7.22 ± 0.07	5.37 ± 0.32	2.89 ± 0.26	ND
BSD1-I	14.36 ± 0.42	2.86 ± 0.25	12.99 ± 0.72	ND
BSD2-I	9.11 ± 0.56	2.78 ± 0.14	13.14 ± 0.94	ND
BSD3-I	7.97 ± 0.37	3.38 ± 0.25	23.91 ± 1.37	ND
BSD1-U	12.42 ± 0.28	2.36 ± 0.17	5.06 ± 0.13	ND
BSD2-U	7.41 ± 0.85	4.75 ± 0.28	2.75 ± 0.38	ND
BSD3-U	8.09 ± 0.22	2.82 ± 0.14	24.29 ± 0.81	ND
BSD1-T	13.32 ± 0.45	6.43 ± 0.33	14.97 ± 0.07	ND
BSD2-T	8.18 ± 0.96	9.46 ± 0.21	16.95 ± 0.14	ND
BSD3-T	9.34 ± 0.11	4.13 ± 0.13	11.45 ± 0.36	ND
Mean cookies	6.12 ± 3.37	3.84 ± 1.18	8.11 ± 3.98	-
Mean crackers	9.14 ± 2.25	3.31 ± 0.98	11.24 ± 7.12	-
Mean digestive	9.61 ± 2.14	4.41 ± 1.67	11.27 ± 6.67	-
Mean Spain	6.76 ± 2.11	4.76 ± 1.23	3.12 ± 0.81	-
Mean Iran	9.92 ± 3.08	2.94 ± 1.09	15.35 ± 2.12	-
Mean UAE	7.81 ± 2.23	3.41 ± 1.34	8.13 ± 3.04	-
Mean Turkey	8.63 ± 1.98	4.96 ± 1.15	14.34 ± 1.13	-
EU Limit (Reg. 1881/2006)	-	-	200	100
Significance of variables and interactions				
"Biscuit type"	NS	NS	NS	-
"Country"	NS	NS	$p < 0.05$	-
"Biscuit type*Country"	NS	NS	NS	-

BSC: cookie; BSD: digestive; BSCr: Cracker; S = made in Spain; I = made in Iran; U = made in the United Arab Emirates; T = made in Turkey; ND = not detected; NS: Not significant. Values are expressed as mean ± standard deviation ($n = 3$).

The differences between samples may be related to raw ingredients such as flour, manufacturing operations such as baking, packaging with nylon wrappers, and contamination from the environment, all of which are plausible variables thought to impact the contamination of these biscuits. Other possible sources of variability within and among brands from the same manufacturer include batch–batch variances in manufacturing, differences in brand production processes, and environmental contaminations [4]. Wheat flour is a common ingredient in making biscuits, and as such, plants could absorb heavy metals from the soil [64].

The mean level of zinc detected in the samples varied from 1.46 to 14.36 mg/kg, without a significant effect by the variables "Biscuit type" and "Country" or by their first-order interaction. The observed range was lower than the zinc concentration reported by Arigbede et al. [65], but similar to the findings of Harmankaya et al. [66], who found zinc concentrations ranging from 0.19 mg/kg to 8.62 mg/kg in Turkish biscuits, and of Šebečić and Vedrina-Dragojevic [67] who detected zinc in the range 5.89–17.64 mg/kg in Croatian biscuits. Copper ranged from 1.41–9.46 mg/kg, without a significant effect by the variables "Biscuit type" and "Country" or by the interaction "Biscuit type*Country". The observed copper concentration was higher compared to those of other authors who found 1.15–2.79 mg copper/kg in Croatian biscuits [67]. Zinc and copper are inorganic nutrients known to be higher in biscuits containing whole meal flour [67]. Moreover, wheat bran is known to contribute significantly more zinc compared to rice and oat bran, while oat fiber contains more copper than wheat and rice fiber [68]. Therefore, our findings could be explained by the presence of whole meal flours or bran in some samples (Supplementary Table S1).

Among heavy metals, lead ranged from 2.07 μg/kg to 29.97 μg/kg and was significantly influenced ($p < 0.05$) by the "Country" variable, while the "Biscuit type" variable

and the "Biscuit type*Country" interaction did not exert a significant effect. Biscuits made in Spain showed the lowest lead content. The observed lead content was lower than the levels ascertained by Adimula et al. [69] in 114 snack samples consumed in Nigeria and by Oyekunle et al. [70] who analyzed six popular biscuit types consumed in the same country. Cadmium was not detected in any of the biscuit samples, in agreement with Gopalani et al. [71], who reported that cadmium is absent or extremely low in biscuits from India and Greece [72]. In biscuits marketed in Egypt, instead, the cadmium content was found to reach 0.12 mg/kg [23].

The highest permitted levels of lead and cadmium for cereal grains (not specific to biscuits) are ruled by the EC Reg. 1881/2006 [73], in agreement with the limits set by FAO/WHO [74]. These limits account for a maximum of 0.10 mg/kg for cadmium, and a maximum of 0.20 mg/kg for lead. The US Food and Drug Administration (FDA), instead, set the permitted limit of 1 mg/kg for food primarily eaten by children [75]. All the examined biscuits showed lead and cadmium levels considerably below the maximum allowed limit set by the European Community, as well as below the limits ruled by the WHO/FAO and US FDA.

3.5. Microbial Content

The microbiological features of the examined biscuits are shown in Table 5.

Table 5. Microbial load (log cfu/g) of biscuits marketed in Basrah, Iraq.

Biscuit Type	Total Plate Count (TPC)	*S. aureus*	*Salmonella*	*Bacillus* spp.	Yeasts and Molds
Cookies					
BSC1-S	2.62 ± 0.04	ND	ND	1.04 ± 0.02	1.62 ± 0.02
BSC2-S	2.81 ± 0.07	1.07 ± 0.04	ND	1.03 ± 0.03	1.67 ± 0.01
BSC3-S	2.65 ± 0.03	ND	ND	1.01 ± 0.01	1.63 ± 0.01
BSC1-I	2.61 ± 0.03	1.34 ± 0.03	ND	1.20 ± 0.01	1.61 ± 0.02
BSC2-I	2.51 ± 0.01	ND	ND	ND	1.58 ± 0.02
BSC3-I	2.61 ± 0.01	1.21 ± 0.03	ND	1.23 ± 0.04	1.61 ± 0.03
BSC1-U	2.72 ± 0.03	1.04 ± 0.04	ND	1.38 ± 0.04	1.64 ± 0.02
BSC2-U	2.51 ± 0.04	ND	ND	ND	1.59 ± 0.02
BSC3-U	2.91 ± 0.03	1.14 ± 0.05	ND	1.04 ± 0.03	1.71 ± 0.01
BSC1-T	2.13 ± 0.02	ND	ND	ND	1.64 ± 0.02
BSC2-T	2.61 ± 0.02	1.01 ± 0.07	ND	1.07 ± 0.03	1.61 ± 0.01
BSC3-T	2.82 ± 0.01	1.11 ± 0.03	ND	1.11 ± 0.04	1.68 ± 0.02
Crackers					
BSCr1-S	2.68 ± 0.03	ND	ND	ND	1.65 ± 0.01
BSCr2-S	2.66 ± 0.01	ND	ND	1.21 ± 0.03	1.64 ± 0.02
BSCr3-S	2.75 ± 0.02	1.04 ± 0.04	ND	1.02 ± 0.04	1.67 ± 0.01
BSCr1-I	2.57 ± 0.02	ND	ND	1.32 ± 0.02	1.61 ± 0.02
BSCr2-I	2.59 ± 0.01	ND	ND	1.34 ± 0.03	1.62 ± 0.03
BSCr3-I	2.92 ± 0.04	1.14 ± 0.04	ND	1.36 ± 0.05	1.72 ± 0.01
BSCr1-U	4.11 ± 0.03	1.20 ± 0.04	ND	1.25 ± 0.04	2.33 ± 0.02
BSCr2-U	3.90 ± 0.03	ND	ND	1.20 ± 0.03	2.29 ± 0.02
BSCr3-U	2.80 ± 0.04	1.11 ± 0.08	ND	1.25 ± 0.04	1.70 ± 0.01
BSCr1-T	2.57 ± 0.02	ND	ND	ND	1.60 ± 0.03
BSCr2-T	2.72 ± 0.01	1.04 ± 0.03	ND	1.11 ± 0.03	1.65 ± 0.03
BSCr3-T	2.63 ± 0.05	1.23 ± 0.04	ND	1.25 ± 0.04	1.63 ± 0.02
Digestives					
BSD1-S	2.59 ± 0.01	ND	ND	ND	1.61 ± 0.03
BSD2-S	2.87 ± 0.03	1.07 ± 0.05	ND	1.01 ± 0.04	1.70 ± 0.02
BSD3-S	2.69 ± 0.02	ND	ND	ND	1.65 ± 0.01
BSD1-I	2.57 ± 0.04	ND	ND	1.25 ± 0.05	1.60 ± 0.01
BSD2-I	2.63 ± 0.02	1.23 ± 0.03	ND	1.27 ± 0.06	1.61 ± 0.02
BSD3-I	2.62 ± 0.03	1.16 ± 0.03	ND	1.30 ± 0.03	1.64 ± 0.02

Table 5. *Cont.*

Biscuit Type	Total Plate Count (TPC)	*S. aureus*	*Salmonella*	*Bacillus* spp.	Yeasts and Molds
BSD1-U	2.92 ± 0.02	1.14 ± 0.04	ND	1.07 ± 0.03	1.72 ± 0.02
BSD2-U	2.71 ± 0.01	ND	ND	1.11 ± 0.04	1.64 ± 0.02
BSD3-U	3.82 ± 0.02	1.11 ± 0.03	ND	1.11 ± 0.03	2.28 ± 0.01
BSD1-T	4.08 ± 0.04	1.20 ± 0.05	ND	1.14 ± 0.05	2.31 ± 0.01
BSD2-T	3.84 ± 0.03	1.17 ± 0.04	ND	1.17 ± 0.04	2.27 ± 0.03
BSD3-T	2.83 ± 0.03	1.07 ± 0.03	ND	1.25 ± 0.05	1.69 ± 0.02
Mean cookies	2.19 ± 0.24	0.66 ± 0.41	-	0.84 ± 0.08	1.49 ± 0.11
Mean crackers	2.90 ± 0.31	0.56 ± 0.38	-	1.02 ± 0.07	1.75 ± 0.23
Mean digestive	3.01 ± 0.38	0.76 ± 0.34	-	0.87 ± 0.09	1.81 ± 0.36
Mean Spain	2.70 ± 0.21	0.35 ± 0.31	-	0.71 ± 0.04	1.65 ± 0.06
Mean Iran	2.63 ± 0.29	0.67 ± 0.26	-	1.14 ± 0.09	1.61 ± 0.04
Mean UAE	3.15 ± 0.26	0.74 ± 0.35	-	0.91 ± 0.05	1.76 ± 0.06
Mean Turkey	2.91 ± 0.18	0.87 ± 0.33	-	0.93 ± 0.03	1.78 ± 0.05
WFP limit	-	<1	Absent in 25 g	-	<2
		Significance of variables and interactions			
"Biscuit type"	NS	NS	-	NS	NS
"Country"	NS	NS	-	NS	NS
"Biscuit type*Country"	NS	NS	-	NS	NS

BSC: cookie; BSD: digestive; BSCr: cracker; S = made in Spain; I = made in Iran; U = made in the United Arab Emirates; T = made in Turkey; WFP = FAO/WHO World Wood Program [31]; ND = not detected; NS = Not significant. Values are expressed as mean ± standard deviation (n = 3).

The total plate count (TPC) varied from 2.51 to 4.11 log cfu/g, without a significant effect by the variables "Biscuit type" and "Country" or by their first-order interaction. The International Commission on Microbiological Specifications for Microorganisms in Foods (ICMSF) states that a ready-to-eat food product has a good microbiological quality when its TPC is lower than 4 log cfu/g, while the microbiological quality is unsatisfactory when TPC exceeds 6 log cfu/g [76]. High TPC values indicate the presence of a mixed population of bacteria, including spoilage species [77].

The *Staphylococcus* count ranged from ND to 1.34 log cfu/g without a significant effect by the variables "Biscuit type" and "Country" or by their first-order interaction. The yeast and mold count of biscuits, which was found in the range 1.61 to 2.33 log cfu/g, was not significantly affected by the variables "Biscuit type" and "Country" or by the interaction "Biscuit type*Country". The yeast and mold counts, as well as the *Staphylococcus* count, were found in some biscuit samples above the FAO/WHO acceptable limits of 2 log cfu/g and 1 log cfu/g, respectively [21]. This result might be due to poor handling and storage conditions [78].

The population of *Bacillus* spp. ranged from ND to 1.38 log cfu/g and did not show a significant influence by the type of biscuit, the country of origin, or their interaction. FAO/WHO set a maximum limit of 1 log cfu/g limit for *Bacillus cereus* [31]; however, other institutions, such as the Australian New South Wales (NSW) Food Authority, set a higher level (2 log cfu/g) [79]. The presence of *Bacillus* spp. may be due to the common occurrence of endospores in flour and flour-based products, as well as in the bakery environment [80]. Moreover, the occurrence of *Bacillus* spp. in food products could be due to inappropriate processing, incomplete heating, or secondary contamination by equipment and utensils [81].

Salmonella spp. was absent in all the biscuit samples, meeting the limits set by the FAO/WHO [31]. Das et al. [82], instead, found *Salmonella* spp. in biscuit samples, which may come from eggs or milk. No growth of coliforms and *E. coli* was observed in the examined biscuit samples. This result was in agreement with the Emirates authority for standardization and metrology [83].

4. Conclusions

The increase in the consumption of biscuits as popular snacks, especially in developing countries, including Iraq, makes it necessary to survey their nutritional quality and safety, especially considering that these foods are very appreciated by children. This work gives an insight into the nutritional characteristics and the possible presence of contaminants in cookies, crackers, and digestives produced in Spain, Iran, UAE, and Turkey and sold in the markets of Basrah, Iraq. The obtained findings could help producers in evaluating what to improve in their baked goods.

Interestingly, in many cases, the labeled nutritional facts were found not to correspond to the analytically determined values. The demand for reliable and up-to-date food composition data is increasing among consumers because nutrition is a major modifiable determinant of chronic non-communicable diseases, and scientific evidence has supported the view that dietary changes have strong effects on health throughout life, both positive and negative. It should also be noted that nutritional labeling is mandatory, in addition to being a significant tool in the promotion of a healthy diet. As a result, trustworthy and unambiguous information should be freely available and tightly regulated. This study may raise the awareness of producers in paying much attention to the reliability of nutritional labeling, which is fundamental to consumers for their purchase choice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12189065/s1, Table S1. Coding, net weight and list of ingredients of the biscuits collected in Basrah (Iraq) markets.

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