INTRODUCTION

It is very important to know about the nutritional value of the food which is being consumed by people so far. Various forms of malnutrition are the reason of suffering for millions of the people in each corner of the world. As reported by World Health Organization (WHO), 1.9 billion adults are obese whereas, 462 million are underweight (1). According to Gilani Research Foundation Survey carried out by Callup Pakistan showed that 62% of Pakistani population is consuming packed biscuits whereas, other 31% are consuming open bakery biscuits (2). The word biscuit is derived from Latin word “bis coctus”, which means twice cooked. As compared to other processed food biscuits are popular and the reason of its popularity is its low cost and availability in different forms, its variety of taste and last but not the least its longer shelf-life (3). Biscuits are basically contained wheat flour, sugar, milk powder, baking powder and water as its main ingredients whereas, various other ingredients are also combined to form a healthy and
delicious dough to bake biscuits. It does not contain fermented dough just as bread before its baking (4). Biscuits comprise of wheat, they also contain certain amount of fiber, fats, and protein (5). As peanut biscuits were taken for sensory analysis so the aspect for vulnerability of peanut for being attacked by aflatoxin cannot be ignored (6). Most recently deaths caused by aflatoxins were stated in the United Republic of Tanzania during the summer of 2016 (7). Permissible limit of aflatoxin B1 (AFB1) by United States Food and Drug Administration (USFDA) is 20 parts per Billion (ppb) (8). This research has been conducted in Quetta which is capital of Baluchistan to perform proximate analysis of local biscuits and their comparison to branded biscuits. Aflatoxigenic fungi and aflatoxin B1 were also tested in this study to know the toxicological aspect of wheat biscuits containing peanut.

MATERIALS AND METHODS

Bakeries which used to prepare their own biscuits were selected to collect the samples. Every bakery has its own recipe which they follow to bake biscuits. Biscuit samples were collected which were having peanut in the biscuit’s dough or on topping from Quetta. 16 samples of peanut biscuits were collected from local bakeries whereas, 16 samples of peanut biscuits by well-known brand were collected simultaneously. Samples were collected in zip lock bags, and they were labelled with name, date, and place where they were purchased from. Later, they were shifted in the refrigerator in the nutrition and toxicology lab.

All the apparatus which was supposed to be used in process was sterilized in autoclave before handling. Following analysis were performed on samples: proximate analysis, rancidity test, AFB1 detection, and detection of aflatoxigenic fungi. The methods of analysis are given below:

PROXIMATE ANALYSIS

Proximate analysis of bakery biscuits and branded biscuits samples was carried out using Association of Official Analytical Chemists (AOAC) methods (AOAC, 1980) (9). Detail of procedures performed is mentioned below.

MOISTURE

5gm of sample (W1) was shifted in weighed petri dish. Then it was kept in oven at 105 °C. After 12 hours dried sample was placed into a desiccator for 30 minutes and it was weighed (W2). The sample was again shifted in oven for 60 minutes till the time the constant weight was observed (AOAC, 1980). Weigh lost was noted as moisture and the percentage was calculated by the formula which is mentioned below:

\[
\text{Percent moisture} = \frac{W1 - W2}{\text{Weight of the sample}} \times 100
\]

ASH

2gm of moisture free sample was transferred into weighed crucible and then it was scorched over a low burning flame. The scorched sample was kept in muffle furnace for 4 hours at 550-600 °C until it was turned into a greyish ash. The muffle furnace was turned off as temperature reached 200-300 °C. The crucible was then shifted to a desiccator for cooling purpose (AOAC, 1980). The crucible was weighed, and ash was calculated by the following formula:

\[
\text{Percent ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100
\]

ETHER EXTRACTION

Ether extract was calculated by Soxhlet’s continuous extraction apparatus with the help of fat solvent. 5g moisture free sample was taken into dried extraction thimble. The thimble was then fixed in glass jacket which was fixed under the condenser of the extraction apparatus. Already weighted receiving flask of apparatus was taken and around 150ml diethyl ether (40-60 °C) was poured in that. The water and heater, both were turned on and extraction process was taking place at a condensation rate of 90 drops per
minute for 10 hours. After this, sample was removed, and ether was collected in a glass jacket until it was filled with around 20 ml of ether along with extract. The receiving flask was removed and was heated for vaporizing of solvent. The flask was cooled down and weighed (AOAC, 1980). The percentage of fat was then calculated with the given formula:

\[
\text{% Ether Extraction} = \left( \frac{\text{Conc. Of EE}}{\text{Weight of the sample}} \right) \times 100
\]

**PROTEIN**

Crude protein or total nitrogen was evaluated by Kjeldahl method. Sample was being dried in oven (w1) and transferred in long neck Kjeldahl flask. 5gm of catalyst mixture was taken which was containing HgSO4 and K2O4. Then 30ml of concentrated H2SO4 was added in that. The sample was later boiled in a digestion rack and temperature was low in the beginning and later on at vigorous boiling till the content seemed clear. After cooling of the content down the flask, volumetric flask of 250ml was taken and content was diluted with distilled water. 10ml solution was moved to the micro-Kjeldahl distillation apparatus then distilled in presence of 50mg zinc dust and 10ml of 40% NaOH solution. Produced ammonia was collected in a beaker which contained 10ml of 2% boric acid solution containing 2 drops mixed indicator, which had methyl red and ethylene blue in ethanol. After this, distillate was being titrated against standard 0.1 N H2SO4 till the time when light pink end point was reached. The percentage (%) of nitrogen (N) was calculated with the help of formula given below:

\[
\text{Nitrogen}\% = \left( \frac{\text{ml} \times 0.0014 \times 250}{\text{W1}} \right) \times 100
\]

Crude protein % = N% x 6.25 (AOAC, 1980)

**CRUDE FIBER**

Crude fiber is an organic matter, resistant to weak alkaline solution and weak acids. 2g sample was moved in 600ml beaker which was having reflex condenser. Sample was moisture free, and ether extracted sample was initially digested at simmering temperature, which was 80º C, with 200ml of 1.25% H2SO4 for half an hour or 30 minutes. Frequent addition of hot water helped to keep the constant volume of simmering medium. Protein and carbohydrate were hydrolyzed by addition of hot water. Then under the vacuum the contents were filtered immediately. Remaining residue was cleaned with distilled water right after filtration and moved back to the 600ml beaker. Digestion of residue occurred with 200ml 1.25 NaOH simmering solution, right for 30 min. Residue was washed once again and filtered in the same way. The content was then transferred to the oven at 100º C for the purpose of drying to a constant weight. Muffle furnace was used for ignition of dry residue at 550º C for 20 minutes. The loss in weight was noticed as crude fiber (AOAC, 1980). Calculations of Crude fiber was performed with the assistance of the formula given below:

\[
\text{Crude fiber} = \text{Loss in weight on ignition} \times 100
\]

\[
\text{Weight of the sample}
\]

**RANCIDITY**

Titration of oil samples was accomplished with the help of solution (0.01M) sodium thiosulphate (Na2S2O3.5H2O). 5 gm of the sample and 12 ml chloroform had been added to 25 ml beaker and for this step a graduated cylinder was used. After this, beaker was shaken lightly and shifted to a 250 ml conical flask. 18ml of acetic acid (98%) and saturated Potassium Iodide (KI) solution (0.5-1.0) ml were added later. The mixture had been shaken for a minute and then distilled water (30 mL) was added. Until the yellow color of the reactant was almost disappeared, the mixture was titrated with sodium thiosulphate (0.01M). Five drops of starch solution (1%) were also added, which provided a light blue color to the mixture. The mixture was titrated once again until the light blue color discharged (10). Good quality fats and oils will have a peroxide value of zero. Peroxide values >20 shows very poor-quality fats and oils, which normally would have significant off flavors. For soybean oil, peroxide values of 1–5, 5–10, and >10 correspond to low,
medium, and high levels of oxidation, respectively. It is also called peroxide value which is defined as milliequivalents (mEq) of peroxide per kilogram of sample. (11)

Detection of Aflatoxigenic fungi

**POTATO DEXTROSE AGAR (PDA) PREPARATION**

300g potatoes were bought, washed, and thinly sliced. These unpeeled, sliced potatoes were boiled for 30 minutes. Then broth was stained through cheese cloth. 20g dextrose was added. Distilled water was poured in suspension until the final volume reached to 1 liter. 20g agar was added to the potatoes broth infusion and maintained the final pH 5.6±0.2. It was mixed thoroughly and autoclaved for 15 min at 121°C (15psi), psi is the unit of pressure which means pounds per square inch. Agar was cooled down at room temperature. Safety cabinet was used to pour agar in Patri dished. Hands were disinfected with 70% ethanol and while wearing gloves autoclaved petri dishes were being poured. They were left in safety cabinet for 20 min to be settled.

**DILUTION PREPARATION**

Aflatoxin B1 was tested in the Nutrition and toxicology laboratory of CASVAB. 10g of biscuit was taken and grilled with motor and stick and then they were added in 90ml sterilizes distilled water in a flask. It was mixed well with stirrer and labelled as number 1. After that 1ml sample from label 1 was added into a test tube which was labeled as 2, which was containing 9ml of sterilizes distilled water and shaken well in rotatory shaker. Following that, in test tube with label of 3 containing 9 ml of sterilizes distilled water we added 1 ml sample from test tube 2 and shaken well in rotatory shaker. Immediately following, test tube 4 got 1ml sample from number 3 containing 9 ml of sterilizes distilled water and shaken well in rotatory shaker. On the next occasion, test tube 5 containing 9 ml of sterilizes distilled water got 1ml sample from number 4 and shaken well in rotatory shaker. Then tip was discarded in waste bottle.

**PLATING OF SAMPLES**

After these 2 petri dishes were labeled as A and B for each number of dilution and parent sample 1. 1 ml was taken from parent sample 1 and they were spread or streaked on both plates then dropper was discarded. Same process was done with each dilution 2,3,4 and 5 for each sample. Those plates were kept in incubator for 7 days at 28° C and after a couple of days they were being observed each time.

**TLC SCREENING FOR AFB1 DETECTION**

Thin layer chromatography (TLC) is an affinity-based method which is used to separate various compounds from a mixture. Silica gel TLC plates were activated by heating in oven for 1 hour at 110°C. A pencil line was drawn on the bottom plate with the 3 marks on it. 100 µl of sample was spotted with the help of spotter at left hand spot, right hand spot possessed Aflatoxin B1 standard on TLC plates. whereas middle spot or co-spot contained both the sample and standard, so their retention factors (Rfs) can be compared. These plates were kept vertically in mobile phase which contained chloroform+ acetone+ water with the ratio of 88:12:15 v/v respectively. Till 3-4mm depth the solvent was poured in the chromatography tank. Plates were put in the tank and the lid was put on. Once the solvent front has got the top of the plate, lid was removed. Plate was taken out and a line was drawn where the solvent front was. Plates were visualized under ultraviolet (UV) light (short and long wave).

**STATISTICAL ANALYSIS**

All analytical determinations were conducted in triplicates. Standard deviation and mean were calculated. Duncan, t-test, and analysis of variance (ANOVA) were applied. SPSS software was used to analyze the data.

**RESULTS**
Comparison of local and branded biscuit has given in Table I which shows that locally baked biscuits are rich in protein, ether extracts and crude fiber as compared to branded biscuits. Whereas moisture was more in local biscuits side by side value of ash was also high in local biscuits. Less moisture content in branded biscuits shows that these biscuits have longer shelf life than local biscuits. It has been reported that biscuit should have the moisture content within the range of 1-5%. Low moisture content ensures less chances of microbiological spoilage and longer shelf life in case they are not able to absorb moisture from the air (12).

<table>
<thead>
<tr>
<th>Table I. Comparison of local and branded biscuits</th>
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<table>
<thead>
<tr>
<th>Proximate analysis</th>
<th>Local biscuits</th>
<th>Branded biscuits</th>
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<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>Protein</td>
<td>12.01±.43</td>
<td>12.01±.62</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>26.62±1.74</td>
<td>26.62±0.25</td>
</tr>
<tr>
<td>Rancidity</td>
<td>.55±12</td>
<td>.55±17</td>
</tr>
<tr>
<td>Moisture</td>
<td>4.28±.84</td>
<td>4.28±.12</td>
</tr>
<tr>
<td>Ash</td>
<td>.52±15</td>
<td>.52±.02</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>1.25±.26</td>
<td>1.25±.03</td>
</tr>
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</table>

Protein in local biscuit has observed higher in content as compared to branded biscuits. The reason can be usage of less refined wheat by the manufacturer and wheat used by local bakery has high protein content (12). People can take local biscuits if they want more protein intake. A study had protein 14.71 ± 0.10 and fiber value 1.77 ±0.10 near to the values 12.01 ± .43, 1.25 ± .26 observed in local biscuits (13). Reason of high protein was wheat as it has high protein content (12). High protein of local biscuits boosts its importance as alternative food supplement in human and livestock diets (14). A biscuit contains only wheat flour makes the product healthy to use to control postprandial glucose response after meal (15). Protein and crude fiber observed in local biscuits are having close value to the wheat biscuits taken as a control by another researcher 11.02 and 1.05. Protein also helps in building and maintaining body cells (16). Value of protein and fiber in sample to wheat biscuit used as a control are close to the values of protein and fiber of local biscuits (17). Increase in moisture of biscuits may increase fiber content because of its water molecule binding ability and help water retention however, it can prevent evaporation while process of baking (18).

Crude protein has value 6.6% which is near to the Crude protein value of branded biscuits whereas, its ether extract value is closer to the EE value of local biscuits (19). Results of contemporary study are having much closer value with the result of (20).

Higher ether extracts have been recorded in local biscuits. It can be caused by ransom mixing of fats in dough by local bakeries. Stability of the product can be affected by high ether extract content (21)(22). Whereas branded biscuits have measured and pre decided ingredients to be added in dough. Ether extracts of local biscuits 26.62 ±1.74 have the value near to research (23) 27.67 ± 0.15 which they have observed in pure wheat biscuits used as control in the research. As ether extracts supply essential fatty acids, they are useful for customer who demand for high fiber biscuits, and they are also useful for children (16). It also fulfils the need of energy supply of consumers.

Ash and crude fiber were high in local biscuits as compared to branded biscuits. It has shown wheat used by local bakeries was high in fiber and minerals. Ash contents of biscuits were true representation of inorganic elements attained from the organic element’s combustion of sample (24). These inorganic materials were having mineral elements such as Magnesium, Potassium, Calcium, Phosphorous and iron which are important to make strong structures also reliable functioning of the body (12). Crude fiber is measurement of quality of indigestible cellulose, lignin, pentose like other compounds of food. It is having less food value though it provides bulk essential for peristatic action in intestine (25). According to specification of food safety and standards authority of India (FSSAI) ash content must not be >0.1%, fat must
not exceed from 1.5%, ash content mustn’t be >0.1% (26). Other researchers have observed that product with high ash content could be a good source of minerals (27,28).

Moisture, ether extracts and protein content 3.00%, 21.93% and 7.08% have observed in control biscuits were close to the values observed in brander biscuits but its value of crude fiber was close to the value of local biscuit (29). Moisture of local biscuits and fat contents of branded biscuits were closer to the moisture and fat content of another research (30). In table II significance among samples of local and branded biscuits has shown. p<0.05 has observed which has shown that there was significant difference in protein, ether extract, moisture, and ash among local biscuits. On the other hand, there was no difference observed in protein, crude fiber, ash, ether extract, moisture, and rancidity in branded biscuit samples.

Table II. Significance in branded and local biscuits

<table>
<thead>
<tr>
<th>Local biscuits samples (ANOVA)</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Protein</td>
<td>0.00</td>
</tr>
<tr>
<td>Ether extract</td>
<td>0.00</td>
</tr>
<tr>
<td>Rancidity</td>
<td>0.41</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.00</td>
</tr>
<tr>
<td>Ash</td>
<td>0.00</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0.73</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Branded biscuits samples (ANOVA)</th>
<th>Sig.</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>0.61</td>
</tr>
<tr>
<td>Ether extract</td>
<td>0.63</td>
</tr>
<tr>
<td>Rancidity</td>
<td>0.57</td>
</tr>
<tr>
<td>Moisture</td>
<td>0.52</td>
</tr>
<tr>
<td>Ash</td>
<td>0.06</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>0.48</td>
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</table>

Table III. Percentage of aflatoxin B1 detected in the samples

<table>
<thead>
<tr>
<th>Level of contamination</th>
<th>&lt;20 ppb</th>
<th>Level of contamination</th>
<th>&lt;20 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local</td>
<td>16</td>
<td>87.5%</td>
<td>12.50%</td>
</tr>
<tr>
<td>Branded</td>
<td>16</td>
<td>100%</td>
<td>0</td>
</tr>
</tbody>
</table>

TOXICOLOGICAL ANALYSIS

No aflatoxigenic fungi was detected in either locally baked biscuits or branded biscuits.

Table III has shown that out of 16 local biscuits samples, 87.5 % had <20 ppb, 12.50% were lying between 20-50 ppb and no samples were lying >50 ppb of AFB1 contamination level. Whereas there was no such contamination observed in branded biscuits which is >20 ppb of AFB1. Biscuit samples were analyzed in Babul city of Iraq by a researcher, and it was found that 26 out of 30 samples (86.7%) were contaminated with less than 4ppb of total aflatoxin whereas, 4 (13.3%) were found positive on total aflatoxin with more than 4ppb. 7.9ppb was the Highest contamination rate observed in biscuits samples (31).

CONCLUSION

There was significant difference among the local bakeries whereas, there was no significant difference observed among branded sample in terms of protein, ash, ether extract, moisture, crude fiber, and rancidity. Local biscuits were more nutritious than branded biscuits as local bakers are not measuring the ingredients in a fix quantity, so ingredients have chances of being increased or decreased. AFB1 contamination was observed in local samples most probably the reason could be contaminated wheat or peanut as they both are vulnerable to AFB1 attack. If local bakers will use fix measurements, fresh and good quality ingredients, product can be improved in term of its quality and taste. As carcinogenic AFB1 can cause serious hazards to human life. Bakers can get awareness if they will be given session about how to avoid AFB1 contamination. Nutritionally rich biscuits can play vital role to combat against malnutrition as they are affordable, easy to get and nutritious. Various other composite flours can also be added in local biscuits to enrich them and to make the taste better. Production of acrylamide during baking of biscuits can be studied which also is an important aspect to be researched.
Conflict of interest

The authors declared no conflict of interest.

Acknowledgement:

The authors are grateful to the director of CASVAB, UoB Quetta for providing research facilities.

References:

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