EVALUATION OF MICROBIAL AND HEAVY METAL CONTAMINANTS IN LOCAL AND IMPORTED PACKAGED BISCUITS AVAILABLE IN ISLAMABAD, PAKISTAN

A thesis submitted to Bahria University, Islamabad in partial fulfillment of the requirement for the degree of B.S in Environmental Sciences.

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ABSTRACT

A set of fifteen locally manufactured and fifteen imported biscuit samples were collected from three different locations of Islamabad. All the thirty samples were quantitively analyzed for their microbial and heavy metal content. A structured questionnaire was devised, and a detailed survey was carried out prior to the sample collection. The survey was the basis of the brands we specifically targeted for the sample collection. First the samples were subjected to the microbial analysis where they were tested for total bacterial count, total coliform count and salmonella and shigella count using Nutrient agar, EMB agar and S.S agar respectively. The results of total coliforms, salmonella and shigella were negative for all the samples as no growth was observed on the EMB and S.S agar plates however certain growth could be observed for total bacterial count for all the samples on Nutrient agar plates. Almost all the samples contained total bacterial count within permissible limits according to the Pakistan standards i.e. 10,000 cfu/g however 7 samples showed uncountable total bacterial count and cannot be deemed safe for consumption based on their microbial analysis. After the microbial analysis all the samples were tested for the presence of nickel, cadmium, chromium and lead. The amount of each heavy metal was detected using Atomic Absorption Spectrophotometry and the results were statistically analyzed using SPSS software. Nickel is the most abundant heavy metal present in all the samples more than the permissible limit as provided by WHO i.e. 0.05 mg/l (ppm) except 2 locally manufactured biscuits that contain nickel in permissible amounts. Seven samples contained chromium more than the permissible limit i.e. 0.06 mg/l (ppm) while lead and cadmium were present in permissible amounts in all the samples.

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ABBREVIATIONS

CFU	Colony Forming Unit
NA	Nutrient Agar
EMB	Eosin-Methylene Blue
E. coli	Escherichia coli
S.S	Salmonella and Shigella
WHO	World Health Organization

CONTENTS

CHAPTER 3

RESULTS AND DISCUSSION

FIGURES

TABLES

CHAPTER 1
INTRODUCTION

1.1 Background

Human health is the most important matter of concern presently. Health is directly associated with the food humans consume on a daily basis, therefore it is important to ensure that the food we consume is enriched with the essential nutrients that need to be consumed in specific amounts and is also free of any harmful contaminants that may cause damaging effects on human health.

Food contamination is a significant issue regarding food safety and security all over the world (Thompson & Darwish, 2019). The rapid growth in world population and rising competition in trade has further aggravated the concerns regarding food safety especially in developing countries such as Pakistan. Food safety is not only a major health concern, but it is also a serious economic concern as food borne illnesses are a source of additional economical losses for a country adding up to the health care costs. Thus, maintaining the quality of food produced while working to sustain and improve the economic status can be a serious challenge for countries that already have poor economies. Currently the World Health Organization considers food safety as a matter of top priority and is making tremendous efforts to address and overcome this issue especially in underdeveloped nations (Akhtar, 2015).

In order to overcome the risks and hazards associated with food contamination, one needs to have a proper understanding of how food can get contaminated through different sources. There are many potential toxic substances present in the environment, organic as well as inorganic, that can directly or indirectly contaminate the food human beings consume daily, through various pathways (Thompson & Darwish, 2019). For example, the use of pesticides and herbicides can directly contaminate agricultural crops, contaminated waterways pose an indirect threat to human health, and mostly in packaged foods contaminants can directly enter the food through the packaging material. Such contaminants are referred to as 'migrants' and they leach from the packaging material into the food source during the processing and packaging period (Thompson & Darwish, 2019).

Every individual depicts varying tolerance and susceptibility towards such contamination depending on his or her age, health condition, consumption patterns, dietary makeup and level of exposure to the contaminants. For example, young children are more susceptible and at a higher risk of exposure because they have

relatively weaker immune systems and they tend to consume packaged foods at a much higher rate than others. The level of toxicity that the contaminants exhibit in an individual depend on the route of exposure as well as the amount in which they are consumed. Also, the nature of the contaminant determines as to why some foods are contaminated more than others because some contaminants are more accumulative as compared to others. Depending on these factors the contaminants can cause short term or long-term toxic effects at different life stages of an individual (Thompson & Darwish, 2019).

1.2 Biscuits: A High Impact Food

Certain foods are consumed at a higher rate by individuals of all age groups than others therefore; they pose higher risks to human health. Biscuits are one of the most popular snack foods along with chocolates, chips and other bakery items as they are readily available at every local shop and supermarket throughout the country. Biscuits are consumed by more than 50% of our population on a daily basis because they are a ready to eat, conveniently accessible and inexpensive product, containing vital nutritious elements and are popularly consumed by individuals of all age groups (Abimbola & Gupta, 2017). Therefore, it is necessary to be aware of any kind of contamination that maybe present in such a significantly consumed snack food.

The common ingredients of biscuits comprise of sugar, water or milk, fats, flour and additional flavorings (Iwegbue, 2012). These ingredients used to process biscuits, whether they are home-made, or store bought remain the same. Biscuits are mostly processed and produced in factories rather than being a common home-made food and thus, fall in the category of higher risk foods. The reason that biscuits can be considered among the higher risk foods is because the raw materials they are made of maybe contaminated prior to their processing for example, the milk being used may have severe microbial contamination which in turn would cause the biscuits being produced highly contaminated with microbes as well. Additionally, the surrounding environment, machinery being used, packaging material etc are different concerns related to the production and processing of biscuits in factories and maybe contributing factors in their contamination.

1.3 Heavy Metal Contamination

Heavy metals are potential bio-accumulative contaminants that have the capability to cause harm to human health if they are present more than the permissible limits (Salama & Radwan, 2005). While most of the heavy metals are known to cause serious bio-toxic effects in the human body, there are some heavy metals that are considered essential for consumption. This is because such essential metals are constituents of enzymes and some vital proteins in our body and their deficiency could cause obstruction in the metabolic functions, however even these essential metals need to be consumed within specific limits else their excess would cause toxic effects on human health (Adegbola, Adekanmbi, Abiona, & Atere, 2015).

Some of the essential heavy metals include iron, zinc, copper, cobalt, chromium, manganese etc whereas lead, mercury, nickel, cadmium are some of the heavy metals that are considered to be toxic and have the potential to cause adverse effects on human health even in small amounts (Gopalani, Shahare, Ramteke, & Wate, 2007). Heavy metals are considered such a serious threat to human health because once they enter in our system, there is no proper mechanism present in the body for their removal.

Therefore, even in trace amounts they can pose life threatening risks to humans (Gopalani et al., 2007). This research focuses on four heavy metals namely lead, cadmium, nickel and chromium. Out of these four, three heavy metals are potential toxins having severe health risks associated with them. Lead and cadmium in particular are cumulative toxins having exceptionally toxic effects on human health (Harmankaya, Özcan, Duman, & Dursun, 2012).

According to the World Health Organization reference value, the weekly intake of lead should not exceed 0.05 mg/kg and 0.075 mg/kg for cadmium. Lead and cadmium are two of the most abundant heavy metals and their excess in food sources is associated with the number of diseases caused by these metals (Salama & Radwan, 2005).

Cadmium is present in the compounds used as color pigments for food colors. Thus, there is a high chance for the presence of this metal in many food stuffs however its concentration varies significantly in different foods. However, in processed foods such as biscuits food colorings are excessively used so there is a potential risk of cadmium toxicity associated with such foods. Cadmium toxicity can

have significant damaging effects on the kidney and skeletal muscles and it is also considered to be a significant human carcinogen (Salama & Radwan, 2005).

Lead can enter the food chain through various sources. Airborne lead particles can be deposited on soil, water and crops and that is how lead enters the food chain, contaminating the food sources. Once it is consumed, lead accumulates in the skeletal muscles causing renal tubular damage and can also damage the kidneys. Lead is also considered to be a potential human carcinogen like cadmium and is associated with many human cancers (Salama & Radwan, 2005).

Nickel and chromium are known to cause cancers in human beings that are exposed to these metals. Nickel occurs in the environment at very low levels. In food items,. nickel is naturally present in small amounts however, chocolate and fats contain very high quantities of nickel. Thus, biscuits have a higher chance of having nickel contamination as fats are the primary ingredient used to make biscuits. Consumption of large amounts of nickel can cause severely damaging effects on human health such as lung, nose, larynx or prostate cancer, respiratory dysfunction, asthma, lung embolism, chronic bronchitis etc are some of the health consequences that one needs to face when suffering from nickel toxicity (Mahurpawar, 2015).

Chromium is a heavy metal that is considered to be one of the essential elements required to be consumed in specific amounts. However, like all other essential metals, it can exhibit toxic effects on human health when consumed more than the required amounts. Chromium can contaminate food sources through soil and water (Mahurpawar, 2015) as chromium particles tend to accumulate in the soil and adhere to sediments present in water. Chromium is present in the environment in different forms. Cr (III) is considered an essential nutrient whereas Cr (McCarthy et al.) is a potential toxin and a known human carcinogen as well. A variety of health risks are associated with chromium toxicity such as different types of ulcers, kidney and liver damage, respiratory dysfunctioning etc (Martin & Griswold, 2009).

1.4 Microbiological Contaminants

Microorganisms are responsible for spoiling and contaminating many food items (Abimbola & Gupta, 2017). Microbial toxicity is an alarming issue when it comes to food safety and security. Most foods are at a high risk of being contaminated with different life-threatening microorganisms whether they are processed in industries or homemade. It is necessary to determine the microbial quality of food in

order to establish whether that certain food is safe to consume or not. Food can get contaminated with pathogenic microorganisms, directly or indirectly, at different stages during the production process (Alum, Urom, & Ben, 2016).

Pathogenic microorganisms are present almost everywhere in the environment and can contaminate food sources via a variety of routes and sources. For example, sometimes pathogens can contaminate food sources via sewerage lines, many pathogens are present in the flesh, milk, eggs and excreta of the infected animals and can indirectly contaminate the food sources, and some pathogenic microorganisms simply exist in the surrounding environment and cause food contamination via a variety of pathways that imitate different ecosystems that constitute our food supply. Prevention of food contamination can be acquired by thoroughly understanding all these different pathways and mechanisms of food contamination (Alum et al., 2016).

There are several health risks associated with different types of microorganisms. For example diarrhea, kidney failure and few other related consequences are associated with *Salmonella*, *Listeria*, *Campylobacter* and *E.coli*. All these microorganisms can be found in many food sources including processed biscuits. Cholera, campylobacteriosis, E. coli gastroenteritis, salmonellosis, shigellosis, typhoid, and brucellosis, are some of the major food borne diseases caused by microorganisms such as *E. coli, Shigella, Salmonella, Campylobacter jejuni, Giardia lamblia, Entamoeba histolytica, Cryptosporidium* etc. Diarrhoe is by far the most common illness associated with foods that are contaminated with microbes and is currently considered a primary health concern in Pakistan (Akhtar, 2015).

Salmonella is one of the microorganisms that could be found in food products such as biscuits. It is a rod-shaped, gram negative, anaerobic bacteria that is a significant food borne pathogen and is dominantly found in poultry, eggs and dairy products. Salmonella infection or salmonellosis is the common disease associated with this bacterium. It is a disease in which the intestinal tract is affected. *Salmonella* is also the cause of diarrhea in some people and in severe cases patients suffer from symptoms such as headache, fever, chills, blood in stool, nausea vomiting etc (Eng et al., 2015).

Total coliforms are another type of microorganisms that are associated with food contamination. Coliforms can be found in soil, water, animal and human waste and can contaminate food sources indirectly through contaminated water. Food that is

contaminated with coliforms poses severe health risks to human health. Some of the diseases associated with coliform contamination include diarrhea, typhoid, Hepatitis A and E etc (Ahmed, Noonari, Magsi, & Mahar, 2013).

1.5 Consumer Purchase Behaviour

Consumer purchase behavior is a study that is used to explain an individual's methods of choosing, utilizing and purchasing a specific product or service in order to fulfill his or her needs (Thangasamy & Patikar, 2014). This is an evaluation that helps to identify what effect a consumer's purchase patterns would have on his or her own self as well as on the society. A consumer's purchase behavior is a combination of all the thoughts, feelings and actions he or she depicts before and while buying any product or service. This helps to specify what an individual is likely to purchase, which brand he or she considers more suitable, where and when the individual would make a purchase from depending on his or her need for a product, price range and quality preference and how many times is the consumer likely to make a purchase. This is a way to have an insight about a consumer's overall purchase decision. The result of this evaluation is a consumer's ultimate decision regarding product and brand preference, purchase time and frequency, and retailer choice (Khaniwale, 2015).

1.6 Objectives

1. To evaluate the extent of microbial contamination present in the selected biscuit samples.
2. To identify and quantify the selected heavy metals in sampled biscuits.
3. To assess the purchase preferences of selected biscuits among consumers of Islamabad.

CHAPTER 2

METHODOLOGY

2.1 Sampling Location

The samples were collected from different super markets of Islamabad, specifically from Punjab Cash n Carry (G-9), Al-Fatah Mall (F-8) and Shaheen Chemist (I-8).

2.2 Sample Size and Sample Collection

The collection of samples was based on a comprehensive questionnaire survey which was carried out prior to the sample collection. The questionnaire gave us a clear perspective on which brands to target (Adegbola et al., 2015) specifically for buying locally manufactured and imported biscuits. Based on the results of the questionnaire survey, a total of 30 biscuit samples were collected (Pundir & Jain, 2011). Locally manufactured brands consist of Peek Freans and LU while imported brands consist of Tiffany, Cadbury and Mcvities. 15 samples of biscuits were collected from locally manufactured brands and 15 from imported brands.

2.3 Microbiological Analysis

The microbiological analysis of each biscuit was carried out by using the standard pour plate method in order to determine the amount of colony forming bacteria present in the samples and the results were expressed as cfu/g (Yusufu, Netala, & Opega, 2016). Prior to the analysis, all the apparatus was thoroughly washed and was autoclaved, along with all the prepared media, at 121°C for 30 minutes (Emmanuel, Olubunmi, & Atanlogun). Three agars were selected, to determine the total bacterial count, salmonella and shigella count and total coliform count for each biscuit sample. Each agar was poured in 30 sterile petri dishes and incubated at 30°C for 24 hours. Afterwards, 0.1 ml of the supernatant from each sample was poured onto the petri dishes and thoroughly spread. Then the petri dishes were properly sealed, labeled and put in the incubator for 48 hours at 30°C. The entire process was carried out in the laminar flow to prevent any kind of external contamination (Agu & Okoli, 2014).

2.3.1 Apparatus

Electronic weighing scale, Pestle and mortar, Sterile Plastic containers, Spatulas, Refrigerator, Laminar Flow, Autoclave, Incubator, Centrifuge, Flasks, Funnel, Cotton Plugs, Measuring cylinder, Petri dishes, Test tubes, Pipettes and Glass spreader.

2.3.2 Culture Media Preparation

A culture media is simply a medium, to grow different types of microorganism and consists the essential nutrients for microbial growth. For our analysis, three different media were prepared and used to determine specific microbial colonies.

Figure 2.1. Culture media preparation

2.3.2.1 Nutrient agar

The nutrient agar is a general-purpose medium used for determining the total microbial count for different microorganisms. The agar was prepared by mixing 12.6 g of the nutrient agar powder in 450 ml of distilled water and then autoclaved at 121°C for 30 minutes.

2.3.2.2 Salmonella and Shigella agar

The salmonella and shigella agar is used for determining the *salmonella* and *shigella* count. The agar was prepared by mixing 23.4 g of the agar powder in 450 ml of distilled water and then autoclaved at 121°C for 30 minutes.

2.3.2.3 Eosin methylene blue agar

The EMB agar is used for determining the total coliform count. The agar was prepared by mixing 15 g of the EMB agar powder in 400 ml of distilled water and then autoclaved at 121°C for 30 minutes.

2.3.3 Sample Preparation

First, 0.9 mg of NaCl was mixed with 100 ml of distilled water to prepare the normal saline solution, which was autoclaved at 121°C for 30 minutes. 10 ml of this normal saline was poured in 30 sterile labeled test tubes for each sample. Then, 5 g of each biscuit sample was crushed and put in its assigned test tube. The mixture was then centrifuged for 10 minutes at 1000 rpm to obtain the supernatant (Emmanuel, Olubunmi, & Atanlogun, 2012).

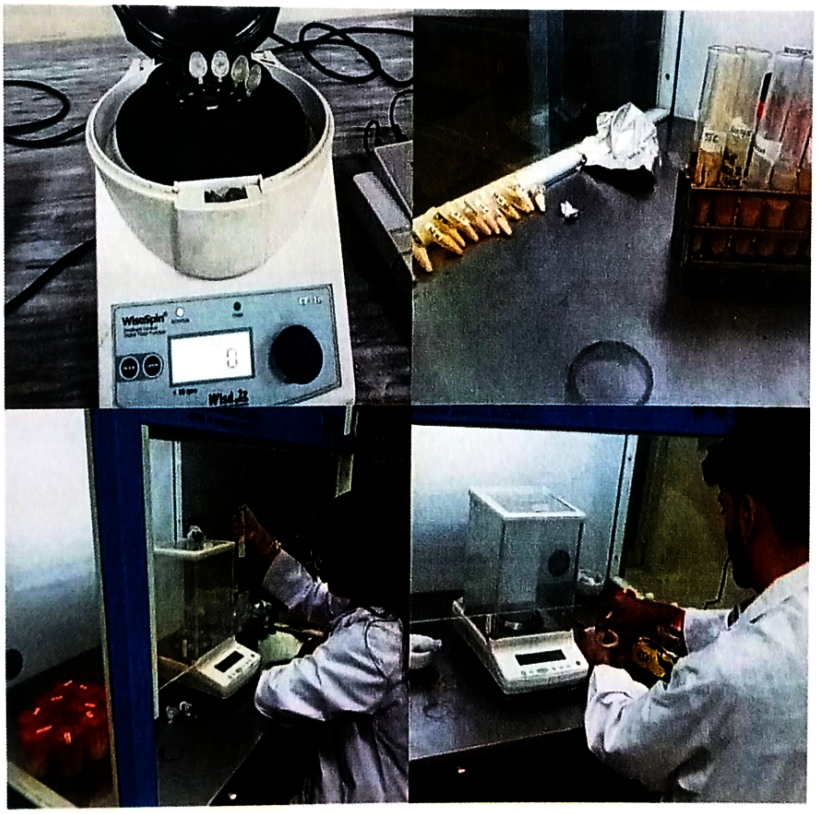

Figure 2.2. Sample preparation for microbial analysis

2.4 Heavy Metal Analysis

The heavy metal analysis of each biscuit was carried out by using atomic absorption spectrophotometry in order to determine the amount of lead, cadmium, chromium and nickel present in the samples and the results were expressed in ppm. Prior to the analysis, all the apparatus was thoroughly washed with distilled water and autoclaved at 121°C for 30 minutes (Harmankaya et al., 2012).

2.4.1 Apparatus

Electronic weighing scale, Beakers, Fume Hood, Laminar Flow, Spatula, Pipette, Hot Plate, Glass stirrer, Measuring cylinder, Funnels, Wattmann filter paper no.42 and Sterile plastic bottles.

2.4.2 Sample Digestion

First, 5g of the crushed biscuit samples were taken and placed into 30 labelled beakers. All the beakers were placed in the fume hood and 10ml of HCL was added in each beaker. Then each beaker was placed on the hot plate and heated at 100+°C for 15min until maximum liquid was evaporated. The beakers were then allowed to cool down entirely, filled with 30 ml of distilled water and the mixture was thoroughly stirred for 5 to 10 minutes until the solid residue and water were completely combined. The final mixture was then filtered twice to ensure that any residue or precipitate was removed and poured into sterile plastic bottles which were labeled for each sample specifically (Adegbola et al., 2015).

Figure 2.3. Sample digestion for heavy metal analysis

2.4.3 Atomic Absorption Spectrophotometry

Shimadzu Atomic Absorption Spectrophotometer (AA-7000 Series) was used for determining the cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) content in each sample. The liquid sample was aspirated into a flame (flame, graphite furnace), where it dissolved and evaporated, and atomization of the analyte occurred. A clean air-acetylene flame was used for all four metals (Adegbola et al., 2015). A hollow cathode lamp produced a wavelength of light which was absorbed by each element and a device called photon multiplier detected the reduction in the light intensity. The amount of light absorbed by each metal showed the amount in which it was present in the sample. The samples were analyzed in triplicates and three separate readings were made for each solution, to avoid any flaw whilst obtaining the exact quantity of each metal. The means of these figures were used to calculate the final quantities of all four metals in each sample (Salama & Radwan, 2005).

Figure 2.4. Shimadzu Atomic Absorption Spectrophotometer (AA-7000 Series)

2.4.4 Statistical Analysis

The data obtained for heavy metals was statistically analyzed using SPSS software version 22. Mean and Standard deviation for each sample was calculated separately and then an overall mean for each metal was calculated to obtain a trend of metal concentration from highest to lowest.

2.5 Consumer Purchase Behavior Survey

A structured questionnaire was devised to analyze the consumer's purchase perception. The questionnaire consisted of three categories each consisting of 4 questions, making a total of 12 questions per questionnaire. The questionnaire was distributed among 150 respondents.

First part of the questionnaire consisted of 4 questions that focused on the respondent's personal information such as his/her age, residential area, per month income and gender.

Second part of the questionnaire consisted of 4 questions that illustrated the respondent's preferences while purchasing biscuits such as whether the respondent prefers local or imported brands, price or quality, homemade or packaged biscuits, and taste or nutritional value.

Third part of the questionnaire consisted of 4 questions that targeted the information regarding a consumer's overall purchase behavior depending on the price range in which the respondent purchases biscuits purchase frequency, the type of biscuit the respondent prefers to eat and packaging preference.

CHAPTER 3

RESULTS AND DISCUSSION

The present study was conducted after an extensive literature review on similar studies relating to the quantitative and qualitative analysis of different food items. In our research, we have carried out the quantitative analysis of different biscuits by evaluating their microbial and heavy metal content (Chowdhury, Khan, Karim, Obaid, & Hasan, 2012).

The study was conducted by first carrying out a detailed questionnaire survey for 3 days. The questionnaires were distributed among 150 consumers which highlighted their anthropometric information, purchase preference and purchase behavior (Homann, Ayieko, Konyole, & Roos, 2017).

The collected samples of biscuits were first subjected to the microbial analysis where the total count of microorganisms, total coliform bacteria, and salmonella and shigella count was detected and the content of these microorganisms was determined in each sample (Hozova, Buchtova, Dodok, & Zemanovič, 1997).

After successfully carrying out the microbial analysis, the samples were further analyzed for the presence of nickel, lead, cadmium and chromium and their concentration is expressed as mg/l (ppm) for all the biscuit samples (Singh, Sharma, Agrawal, & Marshall, 2010).

3.1 Microbiological Analysis

Bacteria are known to cause spoilage and contamination of food items that can lead to severe health problems such as food poisoning, stomach ulcers etc. Earlier studies have shown the presence of pathogenic bacteria and carcinogenic aflatoxins in many bakery products including biscuits (Pundir & Jain, 2011).

According to Pakistan Standards and Quality Control Authority, there are specific standards for biscuits provided by the agriculture and food division. The acceptable limits of microorganisms for biscuits provided by this authority are displayed in table 3.1 (Standards, 2014).

Table 3.1. Microbiological acceptable limits for biscuits (Standards, 2014)

S.No.	Evaluation Criteria	Accepted Standard
1	Total Microbial Count	Less than 10000 cfu/gms
2	*Coliform*	Less than 10 cfu/gm
3	*E.Coli*	Less than 3 cfu/gm

4	*Shigella & Salmonella*	Absent/25 gms

According to the standards provided in table 3.1, the results of our evaluation were positive for total coliform, salmonella and shigella as no growth was observed till 72 hours and the agars were checked after every 24 hours for any microbial growth. This means that all the samples were free from any coliform, salmonella and shigella contamination (Soni, Jatav, & Gupta, 2013).

However, growth was observed on the nutrient agar after 24 hours. While most of the samples showed relatively less total bacterial count, with less than 10 cfu/g and 20 cfu/g, seven samples showed uncountable total bacterial growth, i.e. more than 10,000 cfu/g (Standards, 2014) thus we consider them unsafe for consumption (Hozova et al., 1997).

Table 3.2. Results of microbiological analysis

Sample No.	**Biscuit**	**Nutrient Agar (Cfu)**	**Total Coliform Count (Cfu)**	**S.S (Cfu)**
S1	LU Tuc	58	0	0
S2	Peek Freans Sooper	2	0	0
S3	Peek Freans Gluco	70	0	0
S4	LU Gala	7	0	0
S5	LU Wheatable	1	0	0
S6	Peek Freans Saltish	uncountable	0	0
S7	LU Candy	uncountable	0	0
S8	McVitie's Digestive	7	0	0
S9	Peek Freans Chocolicious	0	0	0
S10	Peek Freans Marie	4	0	0
S11	LU Zeera Plus	482	0	0
S12	Peek Freans Jams Delight	8	0	0
S13	Peek Freans ButterPuff	8	0	0
S14	LU Oreo	1	0	0
S15	LU Prince	67	0	0

S16	Peek Freans Chocolate Sandwich	uncountable	0	0
S17	Cadbury Roundie	4	0	0
S18	Cadbury Crunch Melts	22	0	0
S19	McVitie's Strawberry Creams	uncountable	0	0
S20	McVitie's Ginger Cookies	30	0	0
S21	McVitie's Oat Cookies	55	0	0
S22	Tiffany Crunch n Cream	26	0	0
S23	Tiffany Sugar free Chocolate flavoured Cream	7	0	0
S24	Tiffany Marie	13	0	0
S25	Tiffany Delights	0	0	0
S26	Tiffany Digestive	1	0	0
S27	Tiffany Nutty Bites	uncountable	0	0
S28	Tiffany Chunko's chocochip	uncountable	0	0
S29	Cadbury Chocolicious	9	0	0
S30	Cadbury Caramel Biscuit	uncountable	0	0

The results in table.2 clearly show that total seven biscuits show uncountable total bacterial growth including tiffany nutty bites, tiffany chunko's chocochip, cadbury caramel biscuit, mcvities strawberry cream, chocolate sandwich, saltish and candy.

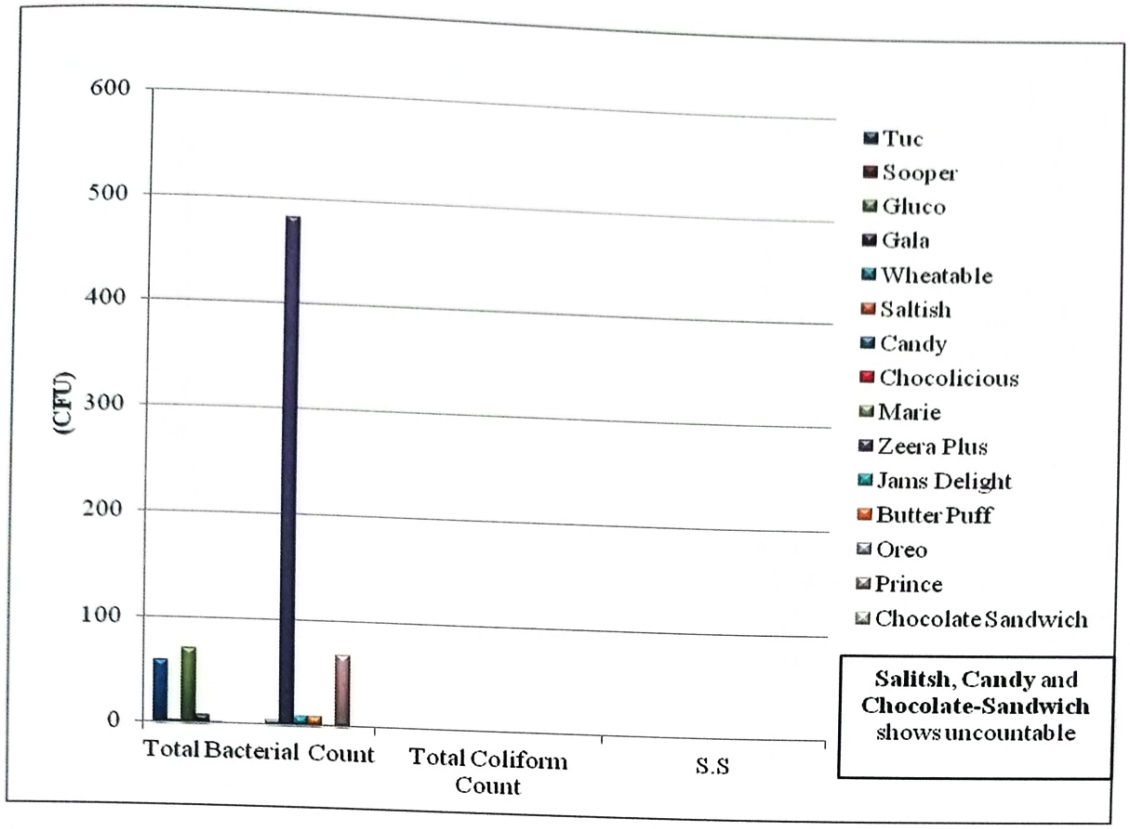

Figure 3.1. Microbiological count in local biscuits

Three out of the 15 local biscuits showed uncountable total bacterial count including saltish, candy and chocolate sandwich which means that these biscuits are not safe to consume owing to the high bacterial content they depict. Sooper, Gala, Wheatable, Chocolicious, Marie, Jam Delight, Butter Puff and oreo depict very less bacterial growth with less than 10 cfu/g. Tuc, Gluco, Prince and Zeera Plus show a higher amount of bacterial growth however they cannot be deemed as unsafe for consumption as the bacterial growth does not exceed the permissible limits of 10,000 cfu/g.

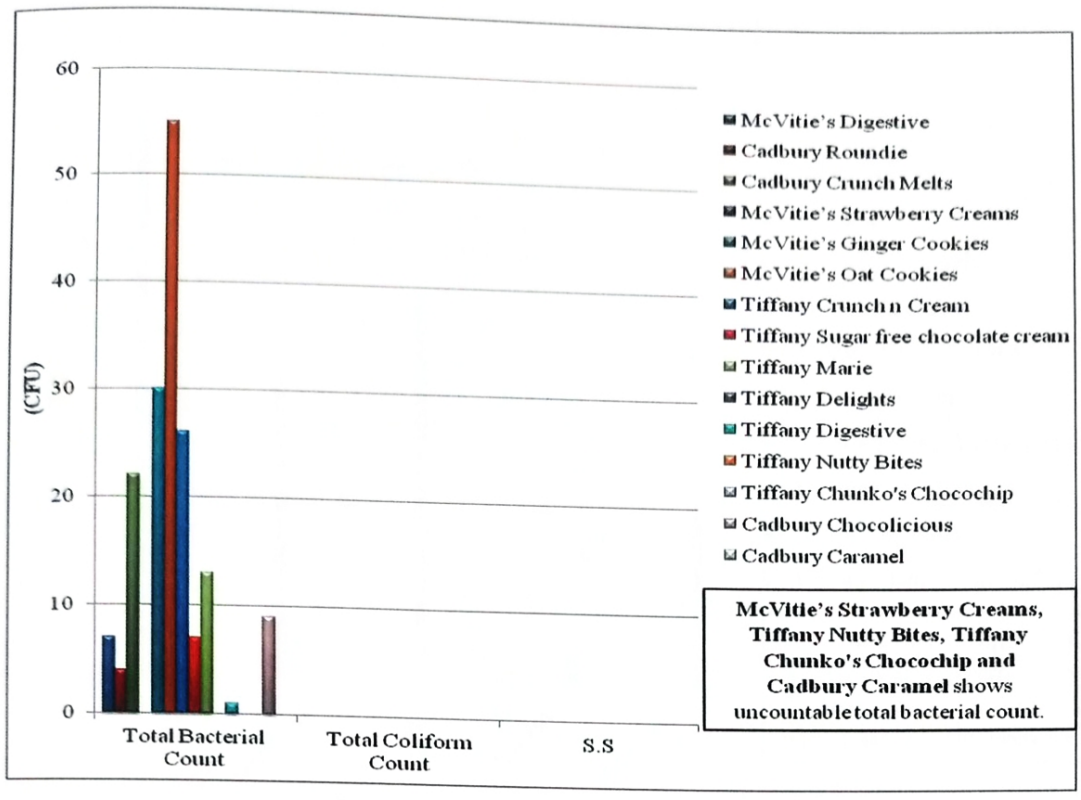

Figure 3.2. Microbiological count in imported biscuits

Four out of the 15 imported biscuits showed uncountable total bacterial count including tiffany nutty bites, tiffany chunko's chocochip, cadbury caramel biscuit and mcvities strawberry cream, which means that these biscuits are not safe for consumption given the high bacterial content they possess, which might contain pathogens (Pundir & Jain, 2011). Cadbury Roundie, tiffany sugar free, tiffany delights, tiffany digestive and Cadbury chocolicious depicted very less bacterial growth with less than 10 cfu/g. The rest of the biscuits showed relatively higher bacterial growth however that is less than 50 cfu/g which makes them safe for consumption.

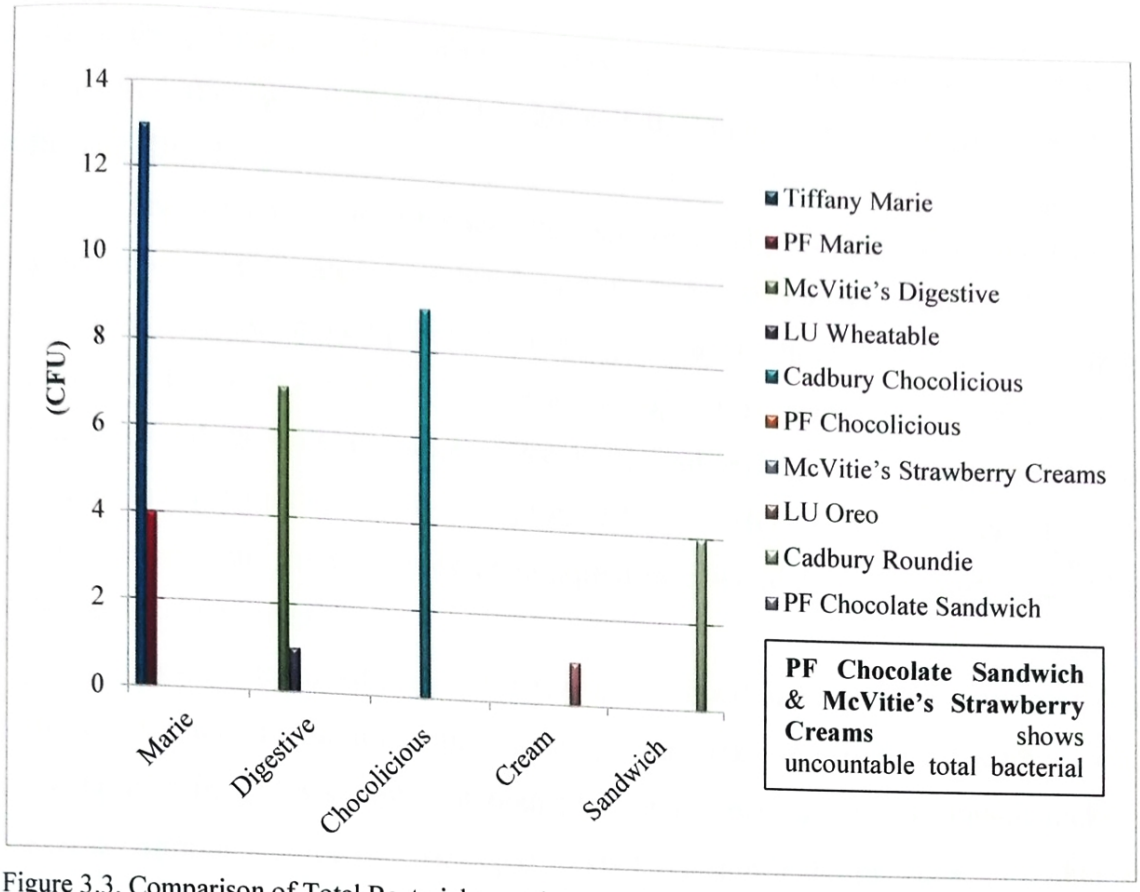

Figure 3.3. Comparison of Total Bacterial count between local and imported biscuits

Comparison between some local and imported biscuits belonging to the same biscuit category show that the local biscuits depict less bacterial growth as compared to the imported brands. However, while comparing the chocolate sandwich biscuit category, it can be observed that the local chocolate sandwich biscuit shows uncountable bacterial growth as compared to the imported Cadbury roundie biscuit. In case of a qualitative analysis, gram staining could be opted for these samples to confirm the presence of pathogenic bacteria but as our study was based on the quantitative analysis only and determining the presence and number of total bacteria in the samples, we did not go for this option (Soni et al., 2013). Most of our samples showed positive results and are safe for consumption based on the results of the microbial analysis.

3.2 Heavy Metal Analysis

Heavy metals are known to cause toxic health effects and inhibit the enzymatic activity if consumed higher than the required and permissible amounts. Some heavy metals are known human carcinogens and can have adverse effects on the liver, kidney and respiratory organs as well(Devi, Bajala, Garg, Mor, & Ravindra,

2016). The permissible limit of nickel, chromium, lead and cadmium as provided by WHO is 0.05 mg/l, 0.06mg/l, 0.1mg/l and 0.03mg/l respectively (Butterworth & Bugang, 2006).

The results of the statistical analysis for locally manufactured and imported samples are represented in table-4 and table-5 respectively. The results of the statistical evaluation compared with the permissible limits of each heavy metal provided by WHO (Koupaie & Eskicioglu, 2015) show that in both locally manufactured as well as imported samples, lead and cadmium concentrations are within the permissible limits as provided by WHO. Three samples of locally manufactured and four samples of imported biscuits contain chromium higher than the permissible limits.

Nickel is the most abundant heavy metal found in all the biscuit samples. Only two locally manufactured biscuit samples contain nickel in permissible amounts while all the remaining 28 samples of both local and imported biscuits contain nickel in excess. Similar studies om packaged foods have shown the presence of nickel and chromium in higher concentrations (Iwegbue, 2012). Different studies conducted by a variety of researchers indicate that there are various sources of metal contamination for packaged foods such as contaminated raw materials, unsafe storage conditions, packaging material and production methods being used (Devi et al., 2016).

Table 3.3. Results of heavy metal analysis

	Ni	**Pb**	**Cd**	**Cr**
Total Biscuits Samples	0.24 ± 0.21	0.04 ± 0.02	0.01 ± 0.01	0.04 ± 0.03
Local Biscuits Samples	0.25 ± 0.25	0.04 ± 0.01	0.01 ± 0.01	0.04 ± 0.03
Imported Biscuits Samples	0.23 ± 0.17	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.03

The mean concentration for each heavy metal is provided in table 3.3. The results show a comparison between the average concentrations of all the heavy metals

present in our samples. It can be observed that the most abundant heavy metal found in both local and imported biscuits is Nickel while the least amount of Cadmium is present in all the samples. The trend of heavy metal concentration appears as Ni> Cr> Pb > Cd.

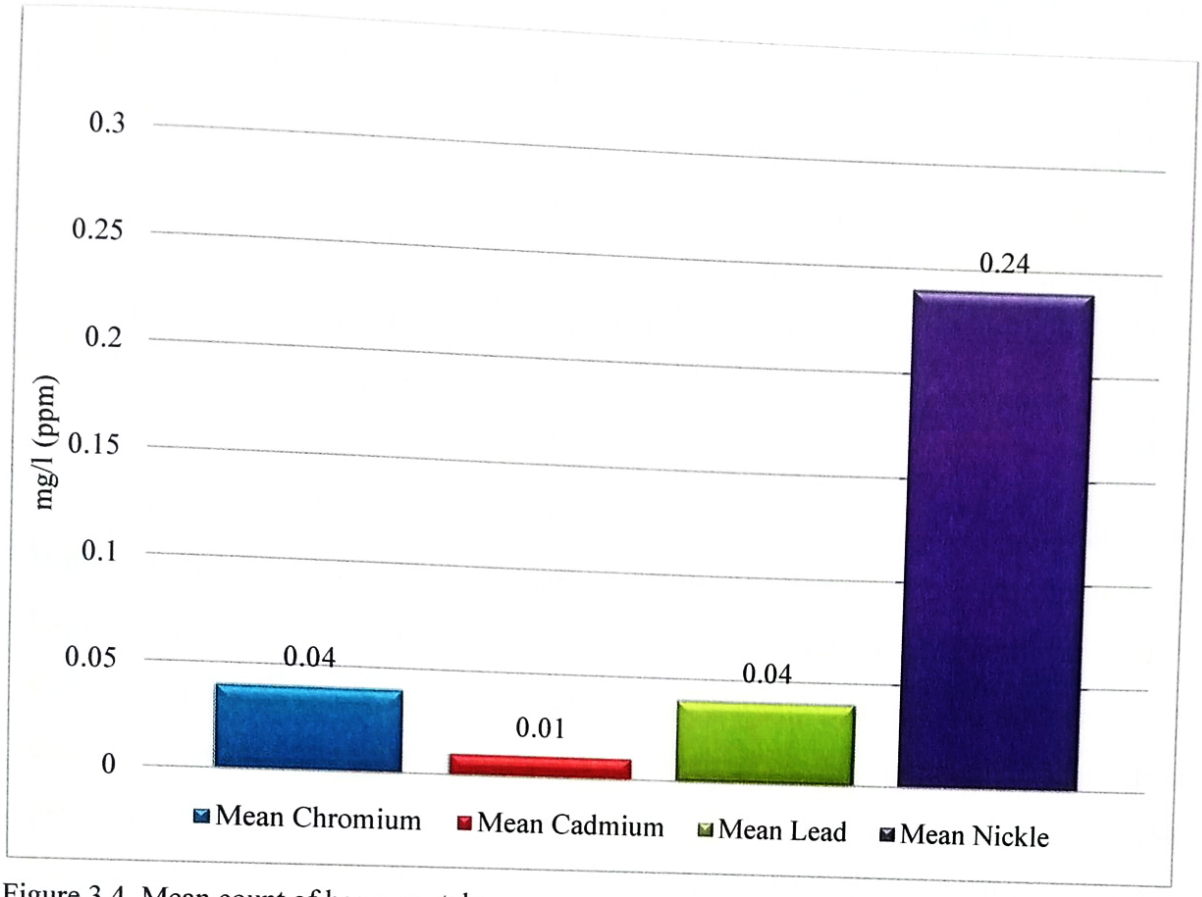

Figure 3.4. Mean count of heavy metals

Figure 3.4 is a graphical representation of the results illustrated in table 3.3 and clearly depict that the mean concentration of nickel is the highest in all the biscuit samples followed by chromium, lead and then cadmium is the least abundant heavy metal present in the samples.

Table 3.4 Heavy Metal Content in Local Biscuits.

Sample No.	**Local Biscuits**	**Heavy Metals** mg/l (ppm)			
		Ni (0.05)	**Pb (0.1)**	**Cd (0.03)**	**Cr (0.06)**
S1	Tuc	0.51 ± 0.33	0.04 ± 0.04	0.01 ± 0.01	0.02 ± 0.03
S2	Sooper	0.05 ± 0	0.01 ± 0.06	0.01 ± 0.01	0.01 ± 0.04
S3	Gluco	0.13 ± 0.06	0.04 ± 0.04	0.02 ± 0.01	0.01 ± 0.04
S4	Gala	0.89 ± 0.59	0.04 ± 0.04	0.01 ± 0.01	0.09 ± 0.02
S5	Wheatable (LU)	0.06 ± 0.01	0.03 ± 0.05	0.02 ± 0.01	0.04 ± 0.01
S6	Saltish	0.33 ± 0.20	0.05 ± 0.04	0.01 ± 0.01	0.05 ± 0.01

S7	Candi	0.16 ± 0.10	0.03 ± 0.05	0.01 ± 0.01	0.02 ± 0.03
S9	Chocolicious	0.08 ± 0.02	0.03 ± 0.05	0.01 ± 0.01	0.09 ± 0.02
S10	Marie	0.10 ± 0.04	0.02 ± 0.06	0.01 ± 0.01	0.05 ± 0.01
S11	Zeera Plus	0.13 ± 0.06	0.03 ± 0.05	0.02 ± 0.01	0.02 ± 0.03
S12	Jams Delight	0.15 ± 0.10	0.02 ± 0.06	0.02 ± 0.01	0.04 ± 0.01
S13	ButterPuff	0.19 ± 0.10	0.06 ± 0.03	0.01 ± 0.01	0.05 ± 0.01
S14	Oreo (LU)	0.33 ± 0.20	0.03 ± 0.05	0.01 ± 0.01	0.02 ± 0.03
S15	Prince	0.05 ± 0	0.07 ± 0.02	0.01 ± 0.01	0.08 ± 0.01
S16	Chocolate Sandwich	0.63 ± 0.41	0.02 ± 0.06	0.01 ± 0.01	0.01 ± 0.04

Table 3.4 shows the permissible limits for each heavy metal as provided by WHO standards along with the concentration of heavy metals obtained in all the locally manufactured samples of biscuits. The results show that only 2 out of 15 samples of local biscuits, Sooper and Prince contain permissible amounts of nickel and the rest of the biscuits contain nickel in excess amounts, higher than the permissible limit. Gala, chocolate sandwich and tuc contain nickel in most abundant concentrations. All biscuit samples contain lead and cadmium in permissible amounts while Galal, Chocolicious and Prince contain chromium more than the permissible limit.

Table 3.5. Heavy Metal Content in Imported Biscuits

Sample No.	Imported Biscuits	Heavy Metals mg/l (ppm)			
		Ni (0.05)	Pb (0.1)	Cd (0.03)	Cr (0.06)
S8	McVitie's Digestive	0.14 ± 0.06	0.02 ± 0.06	0.02 ± 0.01	0.05 ± 0.01
S17	Cadbury Roundie	0.17 ± 0.08	0.03 ± 0.05	0.02 ± 0.01	0.05 ± 0.01
S18	Cadbury Crunch Melts	0.74 ± 0.48	0.05 ± 0.04	0.02 ± 0.01	0.02 ± 0.03
S19	McVitie's Strawberry Creams	0.20 ± 0.11	0.02 ± 0.06	0.03 ± 0	0.02 ± 0.03
S20	McVitie's Ginger Cookies	0.12 ± 0.05	0.05 ± 0.04	0.02 ± 0.01	0.07 ± 0.01
S21	McVitie's Oat Cookies	0.13 ± 0.06	0.04 ± 0.04	0.002 ± 0.02	0.02 ± 0.03
S22	Tiffany Crunch n Cream	0.41 ± 0.25	0.03 ± 0.05	0.02 ± 0.01	0.02 ± 0.03
S23	Tiffany Sugar free Chocolate flavoured Cream Biscuit	0.07 ± 0.01	0.01 ± 0.06	0.02 ± 0.01	0.03 ± 0.02
S24	Tiffany Marie	0.08 ± 0.02	0.06 ± 0.03	0.002 ± 0.02	0.08 ± 0.01
S25	Tiffany Delights	0.17 ± 0.09	0.02 ± 0.06	0.01 ± 0.01	0.09 ± 0.02
S26	Tiffany Digestive	0.31 ± 0.18	0.03 ± 0.05	0.02 ± 0.01	0.07 ± 0.01
S27	Tiffany Nutty Bites	0.19 ± 0.10	0.06 ± 0.03	0.01 ± 0.01	0.05 ± 0.01
S28	Tiffany Chunko's chocochip	0.14 ± 0.06	0.05 ± 0.04	0.02 ± 0.01	0.02 ± 0.03
S29	Cadbury Chocolicious	0.39 ± 0.24	0.03 ± 0.05	0.02 ± 0.01	0.01 ± 0.04
S30	Cadbury Caramel Biscuit	0.14 ± 0.06	0.06 ± 0.03	0.03 ± 0	0.06 ± 0.1

Table 3.5 shows the permissible limits for each heavy metal as provided by WHO standards along with the concentration of heavy metals obtained in all the imported samples of biscuits. The results show that all the 15 samples contain nickel in excess amounts, higher than the permissible limit. Cadbury crunch melts, tiffany crunch and cream, cadbury chocolicious and tiffany digestive biscuits contain nickel in most abundant concentrations. All biscuit samples contain lead and cadmium in permissible amounts while McVitie's Ginger cookies, tiffany marie, tiffany delights and tiffany digestive biscuits contain chromium more than the permissible limit.

3.3 Consumer Behaviour Assessment

The results of the questionnaire survey were analyzed and calculated as percentages which were represented in the form of graphs and tables (McCarthy et al., 2001). The information helped us in selecting the most preferred and consumed brands of both locally manufactured and imported biscuits (Pagès, Bertrand, Ali, Husson, & Lê, 2007). The results of the survey made it clear that the consumption of locally manufactured biscuits is higher than the imported ones.

Table 3.6. Anthropometric Information of Consumer

Age	10-20 years	20-30 years	30-40 years	40-50 years	>50 years
	13.33%	77.33%	3.33%	3.33%	2.66%
Residential sectors covered	G-sector	F-sector	E-sector	I-sector	Others
	30.66%	13.33%	16.66%	6%	33.33%
Per month income	Rs.20-50k	Rs.50-80k	Rs.80-110k	Rs.110k +	N/A
	16%	20.66%	25.33%	34.66%	3.33%
Gender	Male		Female		
	64%		36%		

Table 3.6 shows that 64% of the consumers were males between 20 to 30 years of age. Most of their monthly income was above 1 lac rupees which means that

they can well afford to buy biscuits from imported and local brands on a daily or weekly basis.

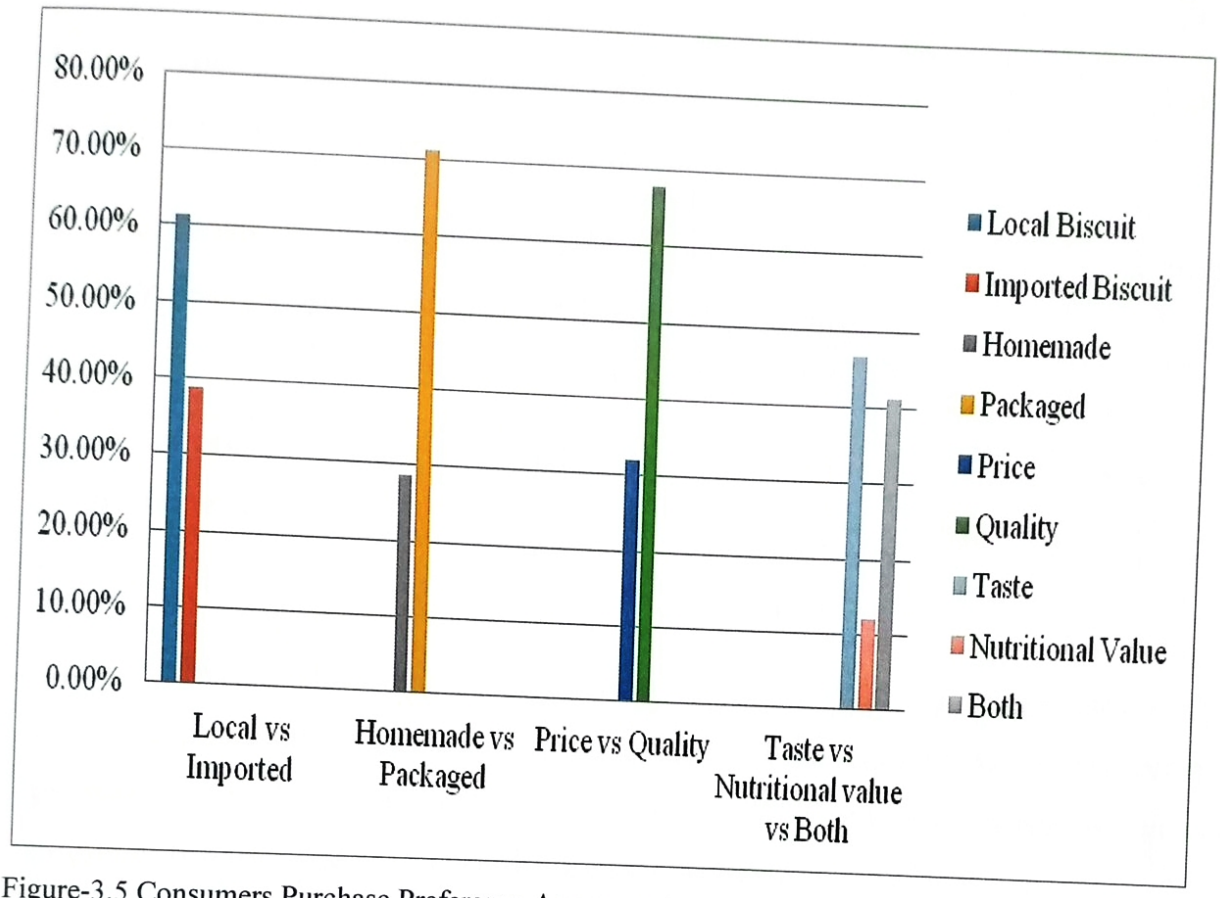

Figure-3.5 Consumers Purchase Preference Assessment

After assessing the consumers purchase preferences it can be observed that 71.3% of the respondents prefer packaged biscuits because it is more convenient to purchase biscuits rather than baking them at home. 61.3 % of the respondents preferred local over imported biscuits, because they are more familiar with the local brands and there is a wider range of local biscuits that is conveniently available for consumers at local shops as well as super markets. 68% of the respondents prefer quality over the price of biscuit and while 41.3% of the respondents prefer both the taste and nutritional value in biscuits, only 12% preferred nutritional value over taste however 46.6% of the respondents said that they would prefer taste over the nutritional value of biscuits.

Table 3.7. Consumers Purchase Behavior

Price range	Rs. 10-30	Rs. 30-50	Rs. 50-70	Rs. 70-100	Rs. >100
	50%	22.66%	6.66%	6.66%	14%
Type preference	Cookies	Digestive	Salty	Cream	Others
	52%	12%	14%	18.66%	3.33%
Purchase frequency	Daily	Once in a 2-3 days	Once in a week	Once in a month	
	16%	20.66%	25.33%	34.66%	
Package preference	Paper wrapped	Cardboard box	Tin box		
	46%	37.33%	16.66%		

Table 3.7 shows the overall assessment of consumers purchase behavior. 50% of the respondents buy biscuits in either half rolls or ticky packs depending on the price range in which they prefer to buy biscuits. Most of the consumers prefer cookie or cream biscuits and prefer paper wrapped packaging.

CONCLUSIONS

The present study confirms that all the 30 biscuit samples were free from salmonella, shigella, and total coliform contamination, however total bacterial count was observed in all the samples among which seven biscuits show uncountable total bacterial growth due to which they are considered unsafe for consumption. The heavy metal analysis for all the biscuits show excess amount of nickel present in 28 samples and 7 samples have excess amount of chromium present in them, however all the biscuits are free from any lead or cadmium contamination.

This contamination can occur through raw materials used for preparing biscuits such as eggs, milk, flour, sugar etc and can also occur during the packaging process as some contaminants are migrants that can travel from the packaging material into the prepared biscuits.

RECOMMENDATIONS

1. Health and Safety authorities should monitor and check food processing industries from time to time.
2. Industry owners should ensure the proper hygiene of their workers and keep a check and balance on the maintenance and cleanliness of the equipment being used.
3. The utensils used during the preparation stage should not be made of any metallic material as most of the metals are used in many alloys, which are used in the containers in which the biscuit batter is mostly prepared.
4. Heavy metals and microbial contaminants can also migrate from packaging material into food stuff by direct or indirect contact hence sterile packaging material should be used that is not printed with a metal-based ink.
5. Raw materials being used for the preparation of biscuits can be the basic source of microbial as well as heavy metal contamination therefore the basic ingredients being used for biscuit preparation should be free from any contamination, properly stored in the optimum temperatures and should be used as soon as possible without being stored for longer durations.
6. People should be aware about the health risks associated with processed foods and minimize their daily intake so that they are less exposed to higher contamination levels.

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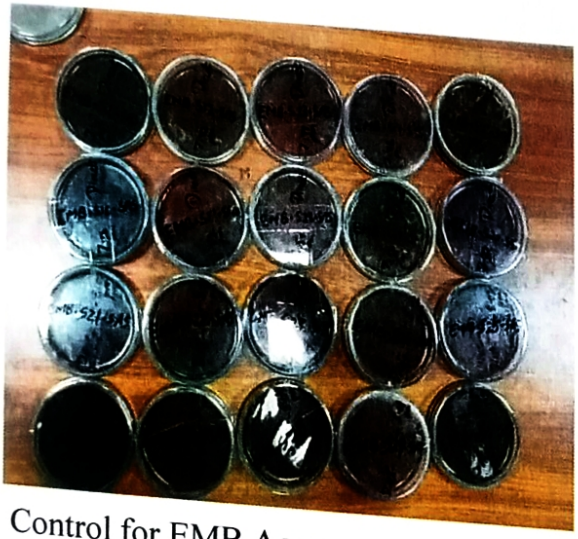
ANNEX

Control Samples before Incubation

Control for S.S Agar

Control for Nutrient Agar



Control for EMB Agar

ANNEX

Control Samples before Incubation

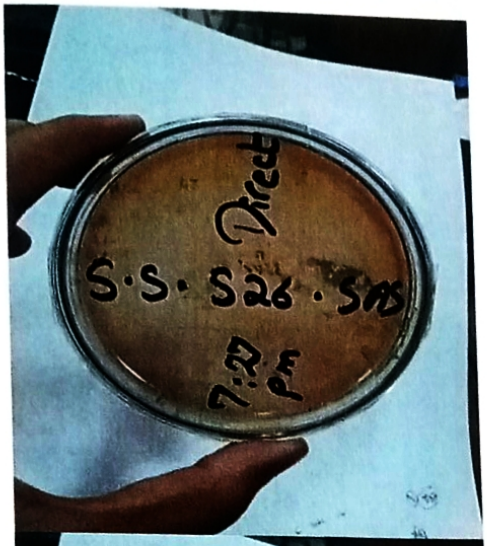
Control for S.S Agar

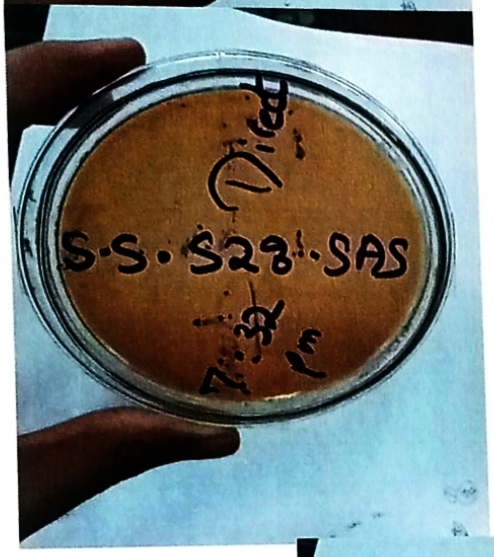
Control for Nutrient Agar

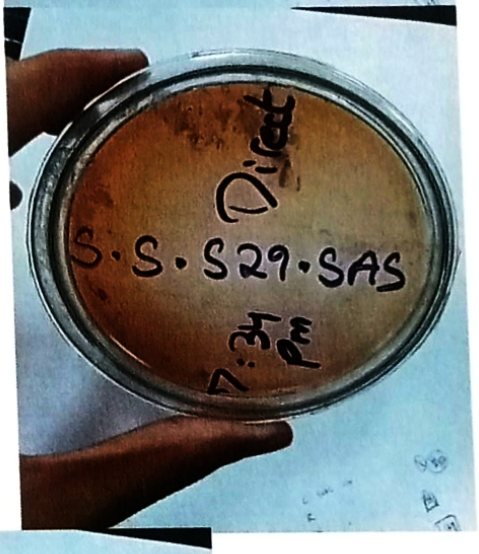
Control for EMB Agar

Samples after Incubation

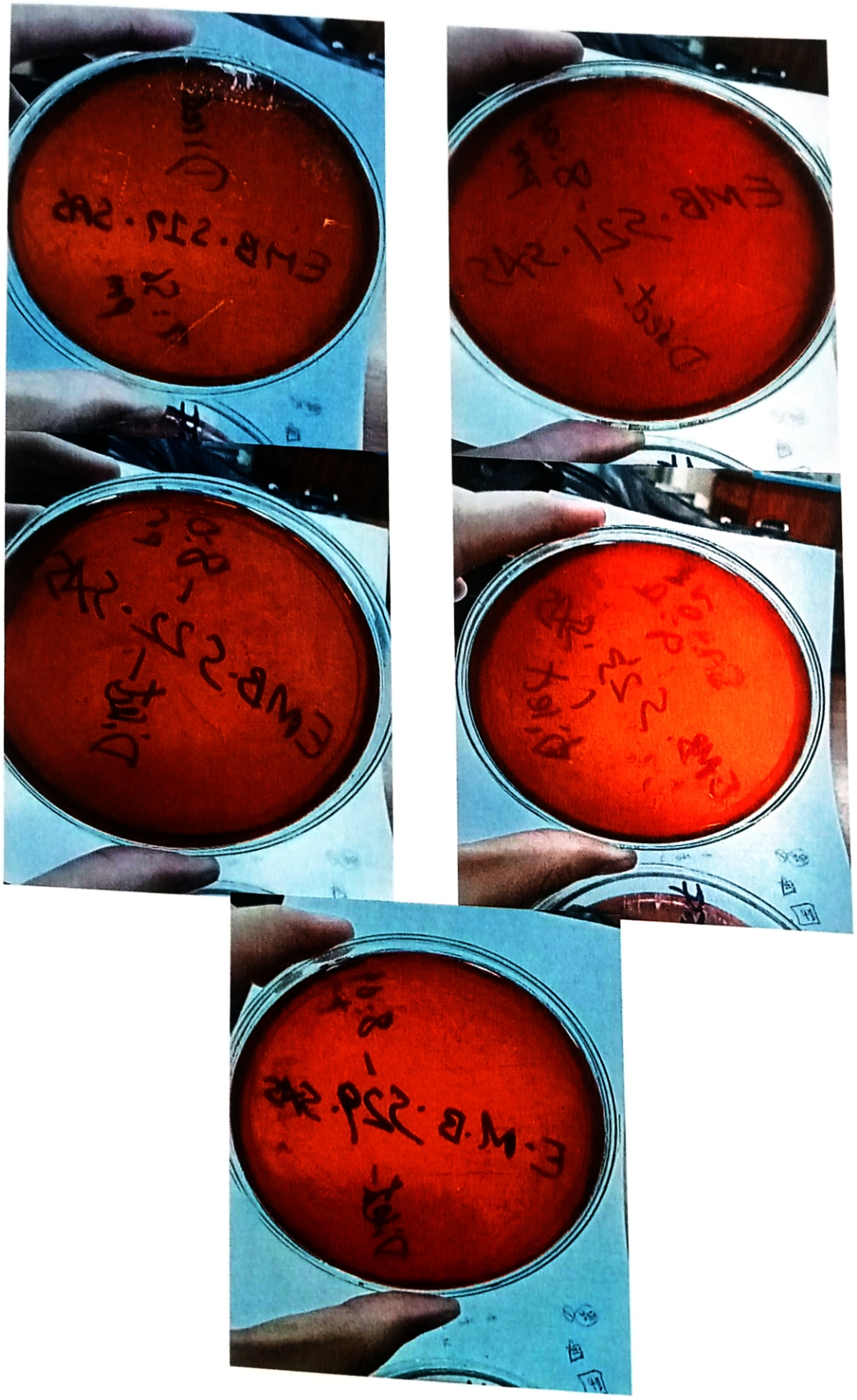
S.S







EMB



Nutrient Agar

QUESTIONARRE SURVEY FOR BISCUITS CONSUMPTION IN PAKISTAN

Dear Consumer,

Good day! This is to assure you that all the information you and your family provide will be treated in the strictest confidence and will not be released in any way which would allow your or your family's privacy to be compromised.

Thank you for your input.

Name of Person Completing Form: _____

Gender: _____

What is your age?

- <10 years
- 10-20 years
- 20-30 years
- 30-40 years
- 40-50 years
- >50 years

Where do you live in Islamabad?

- G-9, G-10 or G-11
- F-6, F-7, F-8, F-10 or F-11
- E-11
- I-8, I-9 or I-10
- Other _____

What do you prefer to have with tea/coffee/milk?

- Biscuits
- Fried Snacks
- Packaged chips
- Other

How often do you purchase biscuits?

- Daily
- Once in 2-3 days
- Once a week
- Once a month

Do you prefer imported biscuits or the local ones?

- Imported
- Local

What is the price range in which you usually buy the biscuits?

- 10 Rs.-30 Rs.
- 30 Rs.-50 Rs.
- 50 Rs.-70 Rs.
- 70 Rs.-100 rs.
- More than 100 Rs.

Do you prefer homemade Biscuits or packaged?

- Homemade
- Packaged

Which brand in biscuits do you prefer to consume mostly?

- Peak Freans
- Tiffany
- Walkers
- LU
- Cadbury
- McVitie's

What type of biscuits you mostly prefer to consume?

- Digestive
- Cookies
- Other
- Creams
- Salty

Do you prefer price over quality?

- Yes, quality can be compromised if the biscuit is in moderate cost
- No, quality needs to be the first priority even if the biscuit is expensive

Which mode of packaging do you prefer?

- Cardboard boxed
- Tin boxed
- Paper wrapped

Do you prefer taste or nutritional value?

- Taste
- Nutritional value
- Both

What is the total income of your family?

- Rs.20,000-50,000
- Rs.50,000-80,000
- 80,000-1,10,000
- 1, 10,000 above

What percentage of people do you believe consume packaged biscuits in Pakistan?

- 20%
- 30%
- 40%
- 50%
- More than 50%

EVALUATION OF MICROBIAL AND HEAVY METAL CONTAMINANTS IN LOCAL AND IMPORTED BISCUITS AVAILABLE IN ISLAMABAD, PAKISTAN

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