MICROBIAL LOAD AND DIFFERENT ADULTERANTS IN COMMON FOOD SPICES (PACKED AND UNPACKED) SOLD IN RETAIL MARKETS IN CHATTOGRAM CITY, BANGLADESH

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Roll No: 0219/14
Registration No: 775
Session: July – December, 2019

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics.

Department of Applied Food Science and Nutrition
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Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

August – 2022

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Sabiha Sultana Mukta

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This is to certify that we have examined the above Master's thesis andhave found that is complete and satisfactory in all respects, and that allrevisions required by the thesis examination committee have been made.

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Dedication

I dedicate this MS research work
to my respected and beloved parents.

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The author

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PLAGIARISM VERIFICATION

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List of abbreviations

Abbreviation	Elaboration
WHO	World health organization
CFU	Colony forming unit
E. coli	*Escherichia coli*
ISO	International organization for standardization
HPC	Heterotrophic plate count
BPW	buffered peptone water
PCA	Plate count agar
API	Analytical profile index
USA	United States of America
XLD	Xylose lysine deoxycholate
BPA	Baird parker agar
PDA	Potato dextrose agar
LCB	Lactophenol cotton blue
HCL	Hydrochloric
SI	Sample identification
SPC	Standard plate count
TCC	Total coliforms count
FC	Fecal coliform
NCS	Not conformed to standard
CS	Conformed to standard
BM	Beef masala
CM	Chicken masala
FCM	Fish curry masala

ChP	Chili powder
CuP	Cumin powder
TP	Turmeric powder
N/D	Not detected

Abstract

Spices are very common food ingredients that are used for food preparation all over the world. The aim of this study was to detect the microbial contamination and screening the presence of different adulterants in common food spices (packed and unpacked). A total of 120 samples of six types (packed and unpacked) of spices (beef masala, chicken masala, fish curry masala, chilli powder, cumin powder and turmeric powder) from retail shops of Chattogram, Bangladesh was investigated to observe the microbiological quality status. Packed spice samples were contaminated free from pathogenic bacteria (normal range- total coliforms <10 CFU/gm and *Salmonella* was 0 CFU/gm) but a total plate count (1×10^2 to 1.7×10^3 CFU/gm (P<0.001)) showed that 66.66% of samples were crossed the normal value of ISO (1×10^3 CFU/gm). Twenty percent (20%) of packed (0 to 170 CFU/gm (P<0.685)) and 91.66% of un-packed (40 to 230 CFU/gm (P<0.531)) spices were crossed the normal range (100 CFU/gm) of mold. Unpacked spices were significantly contaminated with pathogenic bacteria (380 to 1.6×10^3 CFU/gm (P>0.239)) and 83.33% of samples have crossed the limit of total bacteria. Thirty-five percent (35%) of unpacked spices were contaminated by *Salmonella* (P>0.944). The count of *Staphylococcus aureus* in un-packed spices was zero to 430 CFU (P>0.001) per gram of sample. Both packed and unpacked spices were contaminated with different adulterants. Therefore, we strongly recommended that spice manufacturers (for both packed and unpacked) should improve their monitoring system during spices processing, as well as vendors, should include under restriction by government authority.

Keywords: Spices, total coliforms, *Salmonella* spp., *Staphylococcus aureus*, molds, adulterants

Chapter 1: Introduction

Food safety is a serious thinking issue because it can minimize the percentage of illness caused by different foods (WHO, 2015). In many nations, including Ghana, diarrhea is a prevalent food-borne illness, and 16% of children under five dies from it in Africa (Bruce et al., 2005). Many peoples of Bangladesh especially in summer season are affected by diarrhea and cholera which caused by different foods and water. Different types of food, fish, spices are the common source of outbreaks of these diseases (Centre for Disease Control, 2012). Spices are added to foods as food additives to help in food testing (Debs Louka et al., 2013). Because spices have a very low sodium and fat content, their usage has increased (Srinivasan, 2005). However, the environments in which they are grown as well as fundamental processes like drying, harvesting, threshing, shipping, and storage could all result in bacterial, fungal, or insect contamination (Alam-Khan and Abrahem, 2010; Sdecká, 2007). According to Buckenhuskes and Rendlen (2004), this may result in a rise in some food-borne diseases, intoxications, and food deterioration (Ahene et al., 2011). Spice is harmless because it has a low moisture content, but when it comes into touch with some foods that have a high-water content, microbial populations could be boosted due to an increase in water activity (Menlove and Sainsbury, 2002). The majority of production process interventions focus on reducing or eliminating certain contaminants using heat treatment (Zwietering et al., 2016).

Typically, mix-powdered spices are used to grill meat, including chevon, chicken, guinea fowl, suya (dried smoky meat), pork, and many others that are manually processed and sold to the public at various truck terminals, by roadside or itinerant vendors (Mensah et al., 2002). According to Addo's study from 2005, the fungus

count ranged from 3.08 log10 cfu/g to 2.40 log10 cfu/g in the samples of ginger. The sample's total number of aerobic bacteria in the spices ranged from 3.6 log10 cfu/g to 3.7 log10 cfu/g (mixture of ginger and garlic). *Salmonella* spp., *Enterobacter* spp., and *Aeromonas salmonicida* were the bacterial species that contaminated the spice as a whole. After identifying significant levels of *Bacillus cereus*, *Clostridium perfringens*, and *Escherichia coli*, Sagoo et al. (2009) and Shamsuddeen (2009) also reported potential public health danger of spices and herbs. In the local marketplaces, spices are typically sold loose or packaged, and are then consumed in the cities, towns, and rural areas. The phrase "spices" refers to natural dried components or mixes, which includes dried fragrant herbs. The phrase refers to spices whether they are whole, broken, or ground. The root, rhizome, bulb, bark, leaves, stems, flowers, fruits, or seeds of some plants can be used to make these goods. Around the world, spices have long been used to flavor, color, aromatize, test, and preserve diverse meals. In Bangladesh, numerous brands of spices established a sizable market.

In many countries, the foodborne outbreaks of diseases have been a direct or indirect connection to the consumption of food with chili, turmeric powder, and cumin powder (Little et al., 2003). Spices are crucial ingredients of Bangladeshi’s cuisines since ancient times (Das et al., 2012). The sanitary stage and some environmental circumstances and elements of the place they come from, where they are, typically, merely pre-treated and created, determine the microbiological quality of different spice brands all over the world.At every stage of their cultivation to the production line, including harvest, processing, storage, as well as during marketing, distribution, retail, and usage by consumers hands, spices may become contaminated with a variety of harmful microbes (Ahene et al., 2011). Similar to other foods and agricultural products, spices are heavily exposed to environmental microbial contamination during the raw material collection and processing. Contaminated water, dust, animals, flies,

and even human excrement can reach stockholders and retail markets (Trujillo-González et al., 2017).The majority of previous studies on the microbiology of spices have concentrated on and shown the profiles of a few particular microbes, such as total heterotrophs, *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella* spp. , yeast and molds, among others (Baxter and Holzapeel, 1982; Khan et al., 2012; Park et al., 2019; Ei Darra et al., 2021; Mathot et al., 2021; Schmid et al., 2021).In addition to improving the food's shelf life, this control over the microbiological purity of spices before they are sold will lower the health risks, expenses, and losses connected to food alterations during storage (Sun et al., 2014; Mathot et al., 2021). The majority of spices were considerably polluted by spore-forming bacteria, including bacilli of the family Enterobacteriaceae, fungus, and the species Bacillus (Garca et al., 2001; Banerjee and Sarkar, 2003; Witkowska et al., 2011). According to numerous research studies conducted throughout the world, different types of spices sold in Bangladesh are well known for their therapeutic, preservative, and antioxidant properties. (Das et al., 2012; Chakraborty et al., 2020). Numerous countries have conducted in-depth studies on the microbial composition and potential risks to public health connected with the ingestion of different spices, but there is still a disconnect between spice preparation and microbial contamination (Bakobie et al., 2017; Karam et al., 2021).

Adulteration is simply the way of changing the composition of any food item such that it loses the nutritional content. Every food item has certain nutrients which are healthy for the body, but adulteration brings down this value and affects the quality to a large extent. This is the reason that people are getting more prone to health problems. There is no denial to the fact that adulteration is not good. It posed several health hazards which include health diseases, weaken the immune system and lots more. Here are some more harmful effects that you might not be aware of.

There are many mineral oils which when added to the food items can result in paralysis, cancer etc. If pregnant women eat such food items, it might lead to abortion or even damage the brain of the baby. Sometimes zinc substances result in vomiting or in severe cases it can result in diarrhea. Food colors that are added to the items can be the reason of liver damage, allergies, and lots more. Thus, you can say that adulteration can bring down your health and affect the quality of life. As adulteration alters the composition of the food item, it increases the impurities thus making them imperfect for the consumption.

Additionally, when stored in unhygienic settings, spices may come into touch with rodent, bird, and insect feces (Garca et al., 2001; Banerjee and Sarkar, 2004). When added to raw food or after cooking, spices can play a significant role in food contamination under these circumstances, posing substantial health risks. The temperature and vast variation of humidity in tropical nations like Bangladesh may be linked to the contamination of microbes in spices and food in general (Banerjee and Sarkar, 2003; Al Bayssari et al., 2015; Banach et al., 2016). Several reports have examined the manufacturing and quality of a variety of spice items and how they affect human health (Banerjee and Sarkar, 2004; Van Doren et al., 2013).This study's objectives included identifying microbiological contamination and checking for the presence of several adulterants in commonly used food spices (packed and unpacked).

Objectives of the study

1. To determine the total bacterial load, coliform count in spice samples.

2. To determine the presence of *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and molds in spice samples.

3. To detect the different adulterants such as brick powder, Sudan dye III, aniline dye and metanail yellow dye in common food spices.

Chapter 2: Review of literature

Spices are a high-value, low-volume commodity traded on the global market. Spices produce from plants or plant parts that used as condiment for flavoring food in Bangladesh. Spices are not considered as food to the lack of nutritive value though they used in food preparation to aroma or essential oil which considered as the value of spices.

A good number of spices cultivates in Bangladesh and use in most of the food preparation, yet many spices are imported from India, Malaysia, Indonesia. Most of the farmer's land are located behind the different river specially Padma River, a part of vast and fertile region. The~~se~~ river-based areas are flooded often in Bangladesh and blessed with colossal silt to fertilize the riverbed land. Usually, the farmers prepared their lands by the end of rainy season and starts seedling in the beginning of autumn.

Bangladesh is very popular for the spices because it produce~~s~~ different type of spices. In Bangladesh, the most cultivated spices are onion, ginger, red pepper, turmeric, peppermint, coriander, cardamom, cinnamon, cumin, cloves, jayfal, jayatri, dry pepper, and garlic. Spices can be made from a variety of plant parts, including leaves, flowers, fruits, seeds, roots, and rhizomes.

Coriander, dill, fennel, cumin, pepper, chili, vanilla, and anise are obtained from fruits; ginger, angelica, sarsaparilla, turmeric, and asafoetida are obtained from rhizomes; clove, capers, and saffron are obtained from flowers; and cinnamon and cassia are obtained from bark. Basil, coriander leaf, marjoram, peppermint, Indian cassia, and lemon grass are all leaves, whereas mustard, cardamom, fenugreek, nutmeg, and jayfal are all obtained from seeds. The young coriander plant also counted as spice and used as a flavoring and savory for vegetables and fish curry. The most popular spice 'garam masala' is a combination of black pepper, cinnamon,

cardamom, clove that used in preparation of special meat curries and biriany, polao, khichuri. This group of spices (garam masala) also used in the preparation of different foodstuff and sweets.

2.1. Different native spices and their health importance

Ginger: The local name of ginger is Aada. Ginger is used as fresh, raw, or powder form. Usually, ginger use as spice in food preparation, but it has a great medicinal value. It is beneficial for our health. Ginger is an anti-inflammatory spice, which helps to reduce swelling in our body. That may be especially helpful for them who are suffering from Rheumatoid Arthritis and Osteoarthritis. Female, who faces severe abdominal cramp during their period ginger may help. Ginger also helpful to reduce migraine, nausea, vomiting, blood sugar and gastritis.

Cardamom: The local name of Cardamom is Elach. These are very earthy and darkly aromatic. Cardamom use in cooking of different foods and in preparation of sweets and dessert. It also uses in the preparation of raw tea naming ‘masala tea’. Cardamom (*Elettaria cardamomum*), a spice, is also referred to as the "queen of spices."

Cardamom is generally used in special cuisines to prepare different food. It is also used in making black tea. Cardamom has a wide range of health benefits. Now a days cardamom is used to decrease the risk of obesity, depression, bronchitis, influenza and infections. (Singletary, 2022).

Cinnamon: The local name of Cinnamon is Daruchini that is used for the aromatic flavour in food. Cinnamon has been shown in clinical studies to provide important health advantages for people. Cinnamon has unique health benefits, such as anticancer, anti-inflammatory, antidiabetic, and antitumor protection, due to its phytochemical features, such as volatile and phenolic chemicals (Muhammad and Dewettinck, 2017).

Cumin: The local name of Cumin (*Cuminum cyminum* L) is Jira, used in different foods for flavor. It also has medicinal use such as to lose weight. Cumin seeds are used in different food items in both powder and whole seeds form. Cumin has a drastic health benefit on human body. Regular consumption of cumin benefits the treatment of diarrhea, gastrointestinal distress, as well as jaundice, hypertension, childhood maladies, fever, epilepsy, respiratory and gynecological disorders (Singletary, 2021).

Garlic: Garlic is known as Rasun in the area. *Allium sativum* L., a species of bulbous, flowering plant, belongs to the *Allium* genus. It has been used as flavoring of food and traditional medicinal use. It is one of the best researched herbs, which hold a wide range of health benefits. Traditionally it is used to treat cold, infection, heart disease, diabetes, and others health issues. In almost all countries garlic is used to prepare foods for fresh and savory flavors. Regular consumption of garlic is inversely related with the risk of stomach, oral, colon, esophageal and cancers (Chia-Wen et al., 2012).

Garlic has shown the lipid profile lowering effects on patients who are suffer from hyperlipidemia. It also shown that people who are diabetic, regular consumption of garlic and olive oil effectively regulates the serum cholesterol level and triglycerides level (Ansary et al., 2020).

Black Cumin: Kalo Jira is the regional name for black cumin (*Nizella sativa* L.). These are smaller than regular cumin and have a sweet, floral, smoky, and anise-like flavor. Black cumin is a highly valued nutraceuticals herb with a great range of health benefits. The main bioactive compound of black cumin is thymoquinone has the ability to attenuate inflammation and oxidative stress. It also plays role to cell survival, promote immunity, to protect cardiovascular diseases. It also helps to keep

protection against reproductive, renal, hepatic, digestive, neurological disorders, metabolic, cancer and so on (Hannan et al., 2021).

Panch Foron: This Bengali spice blend contains nigella, fennel, cumin, fenugreek, and mustard. Generally, it is used in the preparation of different types of lentils and seeds. Panch foron is a combination of different types of spices and that's why it has a great medicinal value. It is usually used in eastern India in their daily cooking. It was seen that, panch foron has a magical property such as antioxidant, antibacterial, antitumorigenic, antimicrobial, anti-inflammatory, antidiabetic, cardioprotective, and gastroprotective (Sarker, 2019).

Black Pepper: Black pepper is used as powder to prepare different types of dishes. A chemical named Piperine is the major biological active compound in black pepper seeds. The major health benefits of black pepper is, it enhances our digestive tract function. Piperine of black pepper also develops our nervous system. Being enriched with antioxidants black pepper has also antibacterial and anti-inflammatory properties (Singletary, 201).

Turmeric: Turmeric is very common and popular spice in Bangladesh and mostly use in foods for the desirable yellow color. Turmeric is a spice derived from rhizome of the plant, member of Zingiberaceae. Turmeric has tremendous health benefits because of a phenolic compound and a yellow pigment called curcumin. Mainly this curcumin has a great biological action such as anti-inflammatory, antibacterial, anticancer effects (Hay et al., 2019).

Turmeric is usually used to prepare almost in every dish, but it has a great use in Thai, Indian, and other savory dishes. Thai and Indian yellow curry paste is prepared with turmeric and mostly used in different recipes like meat, roast, seafood, vegetables, biryani, soups and so on.

Coriander Leaf: The local name of Coriander Leaf is Dhone pata. It is mostly used in foods as fresh (green leaves). It also called cilantro and it gives flavor. Coriander (*Coriandrum sativum* L), which from Apiaceae family (Mhemdi *et al*., 2011). It is a great source of vitamins, minerals, iron. Coriander leaves is highly enriched with vitamin A and vitamin C. also a great source of zinc, thiamin, and fiber (Bhat et al., 2014).

Coriander leaves has some health benefits like neurological effect, mood enhancer, cognitive improvement, antianxiety effects, pain amelioration, antimicrobial actions (Singletary, 2016)

Mustard Seed: Mustard seed is used in west Bengal for the preparation of hilsha dish called "Sharshe Ilish". It has another use in pickle. Mustard seeds has great medicinal value and health benefits. This contains phenolic chemicals that are antioxidants, such as ferulic acid, 3,4-di-hydroxibenzoic acid, and sinapic acid. Due to the antioxidants and glucosinolates found in mustard seeds, they are employed as a source of bioactive substances in food. Mustard seeds, an effective antioxidant which has the power to protect carcinogenesis. Melatonin, which is abundant in mustard seeds and prevents hypercholesterolemia, (Grygier, 2022).

Lemon: Lemon is used as green and ripen to prepare different items specially in meat to make them more tender and juicier. Lemon is a best source of vitamin C. One of the most significant rutaceae family plants for medicine is lemon. It has anticancer activities and antibacterial effects (kawaii *et al*., 2000). Lemon basically used to prepare different types of dishes specially in meet for juiciness and tenderness. Lemon is also used in cooking for the savory, light and fresh flavor. Additionally, lemon contains a variety of biological properties, including antifungal, antibacterial, antidiabetic, antiviral, and anticancer properties. (Burt, 2004 and Ortuno *et al*., 2006).

Lemon also beneficial who are suffering from constipation, ulcer, gastritis, skin problem, eye problem, and respiratory disorders (Mohanapriya et al., 2013).

Saffron: Saffron is derived from a specific part of the flowers of *crocus sativus* L. One of the priciest spices in the world is saffron. It has numerous uses as coloring agent, preservative, food ingredients, pharmaceuticals and medicinal purposes. Saffron enriches different dishes such as polao in Indian subcontinent, paella in Spain, khoreshes in Iran, others rice items, sea food item and desserts. Saffron is the main ingredients as the key flavoring in Iranian steamed saffron rice with tahdig. Saffron is a source of biologically active compound (crocin, crocetin, picrocrocetin etc). For being enriched with this biologically active phytochemical, a variety of biologically active properties are associated like antidepressant, antioxidational, anti-inflammational, and hypolipidemic actions (Singletary, 2020).

Alzheimer's disease, Parkinson's disease, cerebral ischemia, and depression can all be effectively treated with saffron. Because of its chemical active molecules, saffron also has neuroprotective effects (Sharma et al., 2020).

Other native spices are celery, ajwain, Indian gooseberry, pomegranate seed, basil, almond, rose water, chili, coriander powder, tamarind, nutmeg, mace, curry leaf, cashew nut, fenugreek leaf, lemon, shallot, mint, bell pepper, Indian bay leaf, heeng, fennel seed.

2.2 Common food in Bangladesh and the spices used in the preparation of food

A country of a great cuisine is Bangladesh. Bangladeshi cuisine is a combination of different foods which is mainly influenced by Mughlai cuisine. Also Persian, Arabic, Turkish and many Indian dishes are very popular in our country. The terrain and the country's varied history have influenced all of the food. In Bangladesh the flavor of food is highly valued. For this reason, we use many different spices in a different way

to make the food tasty. A list of common food which are very popular in Bangladesh presented below:

2.2.1 Panta Ilish

Pahela Baisakh is one of the most popular traditional cultures of Bengali people and it comes around the year with thousands of rhymes, poems, and enthusiasm of life. Every year in this day, the Bengali nation organizes various events. Different kinds of food are prepared and exhibited in these events. One of the main dishes in those events is panta-ilish. Panta Bhaat is a popular dish of the rural Bengali community. To make this panta vat, leftover cooked rice is preserved by being soaked in water for a protracted period of time. The next day rice soaked in water is called Panta Bhat. Panta bhaat is eaten by rural people as breakfast mainly. But on the occasion of Pahela Baishakh, panta-ilish is eaten as lunch also.

Panta bhat is usually eaten with salt, raw chilies, dry red chilies and onions, potato fry, brinjal bharta, mashed potato, mashed lentils, shutki bharta etc. The exact history of the connection of Panta-Ilish with Bengali New Year is not known yet exactly. Generally, people knows that Bengali year was established for the convenience of the ruling class. However, welcoming the New Year became a distinctive Bengali culture and custom later. However, during the Mughal rule, members of socio-cultural institutions organized free events, rally and the visiting audience ate the traditional Pantavat. At the end of the 20th century, urban Bengalis started celebrating the Bengali New Year as Pahela Baishakh. On this day Bengalis make it as their traditional food Panta vaat with fried hilsa fish.

2.2.2 Dhakaiya Kacchi Biriyani

Currently, Dhakaia Kachchi is included in the list of famous and traditional dishes of Dhaka. The journey of Katchi Biryani started in Dhaka in 1939. If people go to Old Dhaka, you they can see different kacchi biryani shops to select their food. Different shops used different spices to make this biryani more delicious than others. Here secret kacchi biryani masala is used along with coriander, clove, cinnamon, cardamom, green chili powder, bay leaf, ginger, garlic, onion, cumin powder etc.

The kacchi biryani shops in old Dhaka are always crowded in the morning, afternoon, and night. People from different parts of Dhaka rush there to taste the original kacchi.

2.2.3 Morog Polao

Morog Polao is an ancient traditional dish, which was originated in old Dhaka. At present, Morog Polao is very popular throughout in Bangladesh. Morog Polao is a special rice dish which use chicken with polao rice. Though same rice is used to prepare Polao and biriyani, but their taste is different because of using variety of spices like nutmeg, mace, cumin, aniseed, anise, garlic, turmeric, ginger, coriander, cardamom etc. Morog Polao is not eaten casually, mainly eaten on special occasions and celebrations.

2.2.4 Mezbaani Beef Curry

Chattogram the city of heritage is famous for its traditional cuisine.Mezbani beef-curry is one of them. Historically Mezbani is a traditional regional festival where guests are invited to eat white rice and beef**.** People organize the program of Mezbanon the festival of death and death anniversary, birthday and also on the occasion of naming of a baby, festival of aqeeqa, after the birth of a baby, on the occasion of circumcision of a baby boy, ear piercing ceremony. It also arreanged on

the day of starting of a new business and entrance of a new house. Apart from this, it is also hosted on special occasion or for any auspicious event.

Mezban has a distinct style of cooking that demands a certain mastery of meat quantity. Braised meat cooked with chili and spices. Many spices are used to make this food such as whole cumin, white mustard, coriander, fennel, dried chilies, fenugreek, jaitri, jaiphal, randhuni and poppy seeds.

2.2.5 Chui jhal

Chui jhal, which is one of the most popular and traditional food item. Basically, this name comes from a local name of a stems of the plant of chui (*Piper chaba*), which is used to prepare this item. Chui jhal which is commonly called piper chili, is popularly used for its strongest aroma in Bangladesh, specially in Khulna division. As huge amount of shrimp and prawn produced in this area, it is ~~a~~ very popular in the local people to prepare a shrimp and prawn curry, especially for their guests. They use Chui as a substitute for chili in curries. But chili is also used because chili in curries does not contain as much spicy as chui. People from south-western districts in Bangladesh like Khulna, Jessore, Bagerhat chopped down the roots, stems and then peel off the skin and cut these into small pieces – and cook them with different meat and fish, specially with beef and mutton curry. Chui-jhal along with meat is a traditional cuisine prepared by a combination of chicken, duck, mutton or beef curry. Generally, the following spices are used for the Chuijhal preparation: onion, garlic, ginger, green chilies, garam masala, bell pepper, chili powder, turmeric powder, coriander powder, salt, cumin, cinnamon, cardamom, clove, nutmeg, and Jayatri.

2.2.6 Beef Kala bhuna

Beef Kala Bhuna is a popular tender and dark dish which is prepared with chuck and brisket part of beef and different types of spices. This Beef Kala Bhuna is usually served during Mezban program in Chattogram. Though it is originated in chattogram, very quickly it became more popular across the whole country. Kala Bhuna got its name from its appearance, as ‘kala’ means ‘black' and bhuna means deep frying.

When cooking the meat, which can be either beef or mutton, a broad range of traditional spices are used, along with curd. Various components are used to prepare this pork meal. Onion, ginger, garlic, chili, cumin, coriander, cardamom, cinnamon, clove, chili powder, turmeric powder, black pepper, salt, mustard oil, gorom moshla, nutmeg powder, and toasted cumin powder are among the ingredients for beef or mutton. They are also diced and cubed. Because it is cooked for a longer period of time than other curries, the meat turns black. It also has a distinct flavor from traditional beef curry. Traditional accompaniments for this meal include plain rice, pulao, khichuri, roti, and paratha.

2.2.7 Nihari

Nihari is a stew-based dish made with bone marrow meat and cooked slowly. The Arabic word "Nahar," which means "dawn," is the root of the English term "Nihari". After their morning prayers, Nawabs in the Mughal Empire consumed it as breakfast (Fajr). Others assert that it came from the royal kitchens of Awadh, Lucknow, or Old Delhi during the final years of the Mughal Empire in the late 18th century (modern-day Uttar Pradesh, India).

Beef, lamb, mutton, or goat meat, together with poultry and bone marrow, are the main components of Nihari. But in Bangladesh, beef nihari is incredibly well-known

and well-liked. About 50 different types of spices, including garam masala, cumin, cardamom, and cloves, are used to flavor it.

2.2.8 Shorshe Ilish

Bangladesh is a riverine nation, and the majority of its citizens eat fish every day. Bangladesh produces around 70% of the hilsa fish consumed worldwide, and it is the nation's national fish. The fish is eaten commonly not just in the Indian subcontinent but also in countries like Iraq, Kuwait, Bahrain, Malaysia, Vietnam, Indonesia, and Thailand. There are many ways to prepare it, but shorshe ilish is a favorite among Bangladeshis. Without this fish, any occasion or the menu of a reputable Bengali restaurant would be lacking.

With plenty of green chilies and a splash of mustard oil, slices of hilsa fish are fried in this dish's mustard mixture. White mustard, mustard, mustard oil, green chili, black cumin, turmeric powder, red chili powder, and salt are among the ingredients used in this recipe. For taste, you can include lime juice and/or coriander leaves. There is always hot stemmed rice served with it.

2.2.9 Haleem

The Middle East, Central Asia, and the Indian subcontinent are all major consumers of the traditional, mouthwatering mutton stew known as haleem. This delectable delicacy, which has Persian origins, is today made in Bangladesh with beef, wheat, lentils, spices, and herbs. The meat is mixed or mashed into the curry before being heated up and served with bread. It is a well-liked traditional meal item served at Iftar during the fasting month of Ramadan (breaking fast). It is a well-liked street meal, though, at other seasons of the year.

There are often 4 main ingredients in this slow-cooked delicacy recipe, such as

1. Grain: Wheat or barley is almost always present. Depending on the place of origin of a cuisine, rice and pulses (like lentils) are utilized.

2. Meat: typically consisting of chicken, goat, lamb, or beef.

3. Herbs and spices: a diverse selection that includes garam masala, ground cumin, ground coriander, ground chili powder, ground turmeric, ground black pepper, cilantro, and mint. 4.A liquid for cooking, such as broth, milk, or water. This dish is patiently cooked over a low flame for seven to eight hours, and then it is vigorously stirred. It is a highly filling, healthy, and nutritious dish because it contains meat, lentils, and cereals.

2.3 Rural Foods of Bangladesh and the spice used in

Before the British Raj, Bengal was regarded as India's cultural hub. Under British administration, Bengali cuisine was further altered by Western influences, which the Mughals introduced along with their own cuisine. Bengalis then established their own cooking techniques and culinary tradition.

he national dish of Bangladesh is Bangladeshi food. It alludes to the typical foods and cooking methods used in Bangladesh. It is influenced by Bangladesh's varied history, riverine topography, and climate. There are several rice paddies and a lot of fish in the river in the village area. Fish and rice are hence the main components of Bengali cuisine. In fact, due to their preference for rice and fish, Bengalis are referred to as "Mache Bhate Bangali." For the average Bangladeshi person, rice, fish curry, and lentils are the three most popular traditional dishes.Rural people's meals are seasonal and reliant on what is cultivated because the country is primarily rural and agrarian and more than 60% of the population relies on agriculture. Although the variation is

less, coastal regions will rely more on seafood while highlanders may eat more meat. But practically every dish prepared by Bengali people used a variety of spices.

Meals of rural people

Breakfast: Rural residents typically ate sabzi (mixed vegetables), dal (lentils), paratha (fried flat bread) or Luchi, an omelette, and tea for their first meal of the day. Breakfast in Bangladesh is frequently served with rice and bread, most frequently naan or luchi. In contrast to luchi bread, which is puffed and deep-fried, naan bread is a flatbread made with wheat flour. Roti and paratha are two other bread varieties that rural residents frequently eat. Roti, commonly referred to as chapti, is a less complex form of naan bread. Ghee is used to cook the layers of the paratha, giving it a pleasant and healthful flavor. To cook breakfast meal like vegetables and dal (lentils) different types of spices are used like turmeric powder, green chili, red chili powder, garlic, onion, panchforon etc.

During winter season, breakfast is more enjoyable with various types of Pithas. Rice cakes called pitha can be made sweet or savory using different types of rice, milk, and toppings like sugar, date palm syrup, jaggery, or molasses. Different kinds of spices, such as cardamom, cinnamon, and jafran, are utilized to make these foods more flavorful and delectable.

Main Dishes:

Rice is the favorite dish of every Bengali people. But it can be cooked in different ways like Vuna Khichuri, Patla Khichuri, Jaw Bhaat, Bengali Mishti Pulao (Cashew & Raisin Pilaf), and Chingri Pulao (Prawn Pilaf).

Rice is served with curry, daal, vaji or with the most favorite vorta. Every Bengali love to eat hot steamed rice with vorta. It describes any vegetable that has been

prepared, mashed, and blended with a variety of seasonings, including salt, coriander, onion, garlic, mustard oil, and green or dried chillies. The mashed dried fish dish called Shutki Vorta is highly well-liked in Bangladesh.

In Bangladesh, rice is typically served with curries. They are often made with vegetable oil, a spicy gravy composed of onions, tomatoes, and other ingredients, though in the southern region, coconuts may also be used. All items, including fish, chicken, prawns, cattle, mutton, vegetables, and even eggs, can be prepared in the curry style. Here also used dried chili, onion, garlic, mustard seeds, cardamom, cinnamon, coriander etc to make more tasty and flavoured. Now a days curry masala powder is available in market and it is very popular here.

Bengali loves to eat fish as a main dish. Macher Jhol (fish curry), Shing Macher Jhol, Koi Macher Jhol, Magur Macher Jhol, Mach Vaja (fried fish), Muri ghonto (fish head curry with moong dahl), Ilish Paturi (Hilsa cooked in Banana leaf) are common and popular dishes made with fish. Here they use turmeric, chili powder, garam masala, fish curry masala etc. Jhol is a traditional thin gravy which varies from household to household. It is usually taken in lunch or dinner time. Another Bengali meal made with tiger (bagda) and king (kohli) prawns, coconut milk, and spices is called chingri malai curry (prawn malai curry). Bengalians love this dish, which is offered to guests on important occasions.

Besides fish items, meat (Mangsho) is also popular as a main dish in people. Friday afternoons are synonymous with Mutton Curry and Rice in any quintessential middle-class Bengali household. Meat generally refers to chicken, beef or mutton and usually used quite interchangeably. Mangshor Jhol basically refers to a traditional meat curry cooked in the Bengali style. Here also used coriander, cardamom, cinnamon, turmeric, garlic, ginger, dry red chili etc. to make them savory and delicious.

Dal is a lentil soup common in Bangladesh. Like soup, it is more concentrated. Bengali dal is likewise milder in flavor and contains lentils that have been "mashed," or broken into smaller bits. Along with the onion, potatoes and carrots are occasionally added. Sometimes dal is a combination of spices like garlic, cumin, ginger, turmeric, dry chili and mung beans, often eaten at breakfast with bread. Some types of dal lentils or chickpeas with vegetables and are deep fried as snacks. Without a bowl of dal and rice, no dinner is complete.

Bengali meal is incomplete without vegetables. May be most people believe that Bengalis are all about fish but it's far from the truth. Bengali cuisine has such vegetarian dishes that one might forget about meat and fish. Traditionally, a regular Bengali meal consists of at least two kinds of vegetables a fried vegetable and a slightly curried vegetable. The most popular and delicious vegetable items that belongs to traditional Bengali cuisine are Shukto (mixed vegetable curry), Dhokar Dalna, Sojne Dantar Chorchori, Aloo Posto (Potatoes Cooked in Poppy Seed Paste), Ghoogni, Labra (Mixed Vegetable Sabzi), Lau Ghonto (Bottle Gourd Sabzi), Posto Bora (Poppy Seed Fritters), Mochaar Chop (Banana Flower Croquettes), Jhuri Aloo Bhaja and so on. All these dishes are prepared with a common spice called paanchphoron, which is a Bengali five spice mixture. Equal parts of fenugreek (methi), nigella (kalaunji), cumin (jeera), black mustard (rai), and fennel seeds make up this mixture (saunf).

Snacks items

Snacks are those foods which are taken in small amount between meals. Snacks are eaten with tea in the evening, or any time of the day to kill sudden hunger pangs. Bengali cuisine has two types of snacks - freshly prepared and jar snacks. Whether

eating them with tea or on street walk, these famous Bengali snacks can tantalize any foodie's mind.

The most common snacks in rural areas are Shingara and Samosa. These flaky, little, and filled pastries are simple to prepare during night stops. They are found on roadside stalls. Samosas are triangular-shaped and deep-fried pastries while shingara are rounder flaky. They are made with a hot stuffing of masala potatoes, peas, and green chilies that have been crushed. To the filling, some people might add raisins and cashews. These pastries are deep fried and served with coriander or mint chutney or ketchup. They are made with a crisp coating of maida (refined wheat flour).

Other snacks items that are mouthwatering and appealing includes- Jhalmuri, Pakora, Pua Pitha, Dal er Bora, Beguni, Piyaju, Chotpoti, Fuchka or Pani Puri, Nimki, Narkol Naru, Aloo Kabli, Haleem, Sheek kabab, and Grilled chicken with naan. And to make all of the items tasty a variety of spices are used such as cumin, coriander leafs, coriander powder, chili powder, panchforon etc.

Drinks:

Tea (Cha) is a most popular drink in Bangladesh. It helps to get extra energy or refreshing to the people. The people cannot start our day without a cup of tea. Black tea, green tea, and white tea are just a few of the varieties. In the Sylhet Division of Bangladesh, the seven-color tea or seven-layer tea (Shat Ronga Cha) has emerged as a popular beverage. Each layer differs from syrupy sweet to fiery clove in both color and flavor. To make black tea different types of spices are also used like ginger, bay leaf, cardamom, cinnamon, cloves etc. This black tea is very helpful who are suffering from cold. Also, it contains a lot of antioxidants that’s why it is helpful to protect us from various diseases.

2.4 Adulterants used in spices

Adulterants refer to the contaminants found in different food or food spices that highly risk to food or spices consumers (Jaiswal et al., 2016) The spice industry in Bangladesh is under threat with different adulterants. The spices can be contaminated in any stage of preparation or packaging. Adulterants may also cause health risk to consumers. Rapid screening methods are therefore needed to find fraud and stop it from happening in Bangladesh's sector. Spice-related supply chains frequently span a wide geographic area and are longer, more complicated, and more extensive. These difficulties offer numerous opportunities for evildoers to commit adulteration. The grower, the collector, the primary processor, the local traders, the secondary processor, the exporter, the importer, the trader, the packager, the repackager, the wholesaler, the retailer, and lastly the consumer are the stages of the supply chain. A few opportunities for fraud can arise at any point in the supply chain, including misrepresentation, adulteration, and substitution. Some control measures can be adopted in businesses to identify fraud opportunities or reasons that may arise both inside and outside to the spice industry in order to stop this misleading activity. To maintain safety, cleanliness, and food protection, it is necessary to carefully monitor the entire unit operation during processing and manufacturing. A chance for adulteration to occur early is, during the blending and packaging stage and obviously this stage needs to be carefully monitored. Different types of adulterants are mixed in several spices, like Sudan I, II, III, IV, brick powder in chili powder; Sudan red, metanail yellow, lead chromate on turmeric powder; nut protein, stone dust, semolina on cumin powder; dung powder, husk on coriander powder (Roy et al., 2020).

Azo dyes and triphenylmethanes, which are regarded as hazardous dyes, may be illegally combined with food spices. Sudan I, II, III, IV, orange II, methyl yellow, rhodamine B, and others are examples of these azo dyes.

Triphenylmethane dyes like malachite green and leucomalachite green are examples that are thought to be genotoxic.

2.4.1 Sudan dye III in spices:

Sudan dyes are azo and diazo chemicals that may be widely employed in the spice industry. Despite being prohibited from being in food and food spices, they have been discovered adulterating certain food spices (Fonovich, 2013). These azo dyes can be converted to potentially carcinogenic colorless amines in both the liver of mammals and the microflora found in human skin and the gastrointestinal system. Sudan red dyes have been used to color paprika, chili powder (Dixit et al., 2008). Sudan dyes are not allowed to be mixed to food and food spices since they may have genotoxic and carcinogenic effects on human body. Several procedures, including paper chromatography, HPLC (High Performance Liquid Chromatography), MS (Mass Spectrometry), and several manual laboratory techniques, can be used to determine the presence of Sudan III in chili pepper powder (Daood and Biacs, 2005; Petigara and Scher, 2007). In this study we didn't execute the qualitative test HPLC or MS. To detect the presence of Sudan III in this study we did the manual laboratory test. Maximum large, branded spice industry try not to mix any adulterant to the foodstuff, but the local marketers do it very often.

2.4.2 Aniline dye in spices:

Aniline dye is a dye made by the mixing of aniline, which is a synthetic organic dye. This dye can be used in spice in any processing chain for artificial coloring. But aniline can be toxic if ingested, consumed, inhaled or even by contact with skin. When this aniline is ingested in our body and goes to our blood it can damage a protein that normally transports oxygen to our blood, called hemoglobin. Then the injured hemoglobin can't convey oxygen. If the hemoglobin is damaged and the

damaged hemoglobin cannot be able to carry oxygen to blood, a condition named methemoglobinemia may occur and it is severity depends on how much and how long aniline dye is being consumed (Sourish et al., 2017). Chronic consumption or exposure to aniline dye may cause headaches, anemia, tremor, necrosis, heart problem. In some cases, liver, kidney damage may also occur possibly as secondary effects of hemolysis (Sohini et al., 2020). It can be more harmful for children and lactating mother. On local market retail and wholesaler, repackage can misleads to mix aniline dye as an adulterant in turmeric powder for the color.

2.4.3 Metanail yellow in spices:

Metanail yellow is an organic sodium salt having 3-[(4-anilinophenyl) diazinyl] benzene-1-sulonate as counterion. As a pH indicator in analytical chemistry, this dye is also of the azo class and changes color from red to yellow between pH 1.2 and 3.2. Due of its vivid yellow color, metanail yellow has been employed as an adulterant in turmeric powder. It is not allowed to mix to food and foodstuff as it has neurotoxic and hepatotoxic effect on human body (Dhakal et al., 2016). In turmeric there is a most active compound called curcumin. Curcumin is a potent anti-inflammatory and antioxidant. Having this component in turmeric, it helps to improve symptoms of arthritis, depression, and anxiety. This curcumin has the potential to prevent against cancer and Alzheimer's and improve heart health. Additionally, turmeric powder contains metanail yellow to simulate the appearance of curcumin when the actual curcumin level is low. Metanail yellow is categorized as a CII category substance by the joint FAO/WHO expert committee on food additives toxicity. According to studies done on rats, chronic ingestion of metanail yellow causes hepatocellular carcinoma, tumor growth, neurotoxicity, lymphocytic leukaemia, and has a negative impact on gastric mucin. There are numerous traditional techniques that can be utilized to identify turmeric's metanail yellow. similar to FT-Raman, FT-IR, high

performance liquid chromatography (HPLC), high performance capillary electrophoresis, and iron pair liquid chromatography. But these analytical methods are too much expensive (Dixit et al., 2008). For our financial limitations, we were unable to analyse the samples by the following methods. Therefore, in this study we went for chemical laboratory test to detect metanail yellow in turmeric powder.

2.4.4 Brick powder in spices:

Chili powder can be adulterated with brick powder. Brick powder is added to chili powder to increase the volume as well as to increase the color and weight. Consumption of brick powder in chili powder for long time, consumers could have devastating effects on digestive system. If the chili powder contains red oxide, then it could be carcinogenic for human health (Sen et al., 2017).

2.5. Conclusion

Contaminated foods are highly risk to human health. Foods contaminated with microorganisms can lead different types of gastrointestinal diseases, but different adulterants in food or food spices can cause cancer. Human have right to consume any contamination free food (s) or spice (s). Therefore, food consumers should take aware about their food and food ingredients and government should take some steps to minimize the contamination by regular monitoring.

Chapter 3: Materials and Methods

3. Materials and Methods

3.1. Study Design and Period

The prospective study was designed based on the problem of a current hygiene that is a highly risk to human health, today and future. The study was performed in July, 2019 to June, 2021 to investigate the total count of Heterotrophic Plate Count (HPC), total coliforms, *Salmonella* spp., *Staphylococcus aureus*, and molds and to investigate the presence of different adulterants on the spices.

3.2. Sample collection

A total of six types of 120 different spice samples (60% of each packed and unpacked samples) were analyzed in this study. Samples of the packaged spices were chosen at random from various supermarkets and the unpacked samples were picked up from the street markets in a different region of the Chattogram district of Bangladesh (Table 4.1.1). The packed samples are available in most of the grocery stores in Bangladesh. According to the manufacturer, the products were prepared by the following of all microbiological safety. Six types of spices beef masala, chicken masala, fish curry masala, chili powder, cumin powder, and turmeric powder were used in this study. The samples were brought into the laboratory maintaining 4 °C to check the microbiological quality and each sample was examined in triplicates for this experiment.

3.3 Microbiological Analysis

3.3.1. Total Plate Count

Using a Stomacher lab blender (Seward Medical, London, UK), each sample was weighed out at ten grams and mixed with 90 mL of buffered peptone water (BPW) at a normal speed (1 min for each sample). A serial dilution (1:10) was performed with BPW up to 10^{-5}. Moreover, threefold counting plates were created. Briefly, 0.1 mL of diluted aliquot from each sample (10^{-5} dilution factor) was dropped in the center of Petri plates (10 cm in diameter). Next, 25 mL of plate count agar medium (~55°C) (PCA, Oxoid, No. Cat. OXCM0325B, UK) was added to Petri plates by using 25 mL of the sterile pipette and gently mixed clock and anticlockwise 5 times. For 24 hours, the Petri plates were incubated at 37°C. Colony forming units (CFU) were used to measure the total number of bacteria in each gram of sample.

3.3.2 Total coliform count

The samples were prepared by using a similar protocol previously described in the section of 3.3.1. Brilliance ^{TM}*E. coli*/coliform selective medium (28.1 gm/liter) (Oxoid, No. Cat. OXCM1046, UK) was used to detect coliform and *E. coli*. BPW was used to serially dilute the samples up to 10^{-3}. One hundred microliters of each sample from the dilution of 10^{-1}, 10^{-2} and 10^{-3} were dropped in Petri plates. Next, 25 mL of *Brilliance^{TM} E. coli*/coliform selective agar (~55°C) was added to Petri plates by using a 25 mL of the sterile pipette and mixed clock and anticlockwise 5 times and kept at 44 °C for 48 hours for the incubation. CFU were used to calculate the total *E. coli*/coliform count per gram of material. *E. coli*/coliform strains were further tested to confirm by using the API (Analytical Profile Index, BioMérieux, USA) 20E test. One drop of each inoculum was placed in each well of the API kit. The samples were then

incubated for 24 hours at 44°C, and the color change was used to determine whether the results were positive or negative.

3.3.3. Detection of *Salmonella* sp., *Staphylococcus aureus* and mold

Three selective media XLD, Baird Parker agar (BPA) and Potato Dextrose agar (PDA) were used to grow *Salmonella* sp., *Staphylococcus aureus* (*S. aureus*) and mold. One gram of sample was taken into 9 milliliters(mL) of BPW then kept at 37°C for 24 hours for the preliminary proliferation of microflora. After 24 h of enrichment, the sample was homogenized and then, 0.1 mL of samples were dropped in Petri plates. Next, 25 mL of sterilized XLD medium (~55°C) were added to Petri plates by using a 50 mL pipette and waited until the medium was solid. CFU per gram of sample was used to interpret the results after the plates were incubated at 37°C for 24 and 48 hours. *S. aureus* was inspected on a BPA medium. The samples were prepared by following the similar protocol previously mentioned in section 3.3.1. The results were reported as CFU per gram of sample.

A Potato dextros agar (PDA, Oxoid, No. Cat. CM0139, UK) medium was used to detect the presence of mold in species. Briefly, 10 grams of sample was weighted and dissolved in 90 mL of BPW and homogenized by the following way described in section 3.3.2. Serial dilution was performed with BPW up to 10^{-3}. The Potato dextrose agar medium was prepared according to manufacturer instructions and added extra 5% of chloramphenicol in the sterilized medium (~55°C). Next, 0.1 mL of dilutions were dropped in Petri plates and then, 25 mL of PDA medium was transferred to Petri plates by using a 50 mL pipette and homogenized 5 times clock and anticlockwise. The plates were incubated at 25°C for 48 h and the results were interpreted as CFU per gram of sample. Then, the molds were observed under a light microscope by lactophenol cotton blue staining (LCB) On a glass slide, one drop of LCB was applied

and mold mycelia were transferred to a clean glass slide and mixed by a sterile loop. The glass slide's surface was covered with a coverslip and examined under a light microscope. The positive result showed blue color under the microscope.

3.4. Detection of adulterants

3.4.1. Detection of Sudan Red III in chili powder

From both packed and unpacked samples, A test tube was filled with 1 (one) gram of chili powder, followed by 2 ml of hexane. Then shake well and allowed them to settle down. After separation of clean solution added 2ml of acetonitrile reagent and gently mixed. Appearance of a red color in the lower aceto-nitrile layer indicates positive results means Sudan red III is present and appearance of no red color indicates no Sudan red III is present.

3.4.2. Detection of Brick powder in chili powder

Both packed and unpacked chili powder is collected from local grocery shop. 50 ml of water and two grams of chili powder were combined thoroughly. The quick settling indicates the presence of brick powder and slow settling indicates the absence of brick powder.

3.4.3. Detection of metanail yellow in turmeric powder

In a test tube, 10 mL of water, two to three drops of HCL, and two (2) grams of each type of turmeric powder were added. Next, instant violet color was appeared. The existence of metanail yellow in turmeric powder is indicated by the persistence of the violet color, while the lack of metanail yellow is shown by the weakness of the violet color.

3.4.4. Detection of Aniline dyes in turmeric powder

Two grams of turmeric powder was added into 10ml water and vigorously mixed. Next, 1-2 ml rectified spirit was into the solution. If the yellow color in the rectified spirit immediately vanishes, aniline dye is present; if it doesn't, aniline dye is not present.

3.4.5. Cumin powder:

1 (one) teaspoon of both packed and unpacked cumin powder in a glass of water is taken and eave it for a short while (few minutes). If there are any adulterants, they will float to the top of the drink while the pure cumin spice powder will sink to the bottom.

3.4.6. Detection of Sudan Red III in chicken masala powder

One gram of chicken masala powder was transferred into a test tube and 2ml of hexane was added. The solution gently mixed and leave for settling down. 2ml of acetonitrile reagent was added and well shaken after the clear solution had been separated and transferred to another test tube. Appearance of a red color in the lower aceto-nitrile layer indicates Sudan red III is present and appearance of no red color indicates no Sudan red III is present.

3.4.7. Detection of Brick powder in chicken masala powder

Both packed and unpacked chicken masala powder was collected from local grocery shop. Two grams of chicken masala powder was added into 50 ml of water and vigorously mixed. The quick settling indicates the presence of brick powder and slow settling indicates the absence of brick powder.

3.4.8. Detection of metanail yellow in chicken masala powder

Two grams of chicken masala powder was taken into 10ml water in a test tube, 2-3 drops of HCL were added. Then instant violet color is appeared. The persistence of violet color when the solution diluted with water indicates the presence of metanail yellow in chicken masala powder and the feebleness of violet color indicates the absence of metanail yellow.

3.4.9. Detection of Sudan Red III in beef masala powder

One gram of beef masala powder was transferred into a test tube and then 2 (two) ml of hexane was added. After that, give it a good shake and let it settle.2ml of the acetonitrile reagent is added after decanting the clear solution into a different test tube and shaking it vigorously. Appearance of a red color in the lower aceto-nitrile layer indicates Sudan red III is present and appearance of no red color indicates no Sudan red III is present.

3.4.10. Detection of Brick powder in beef masala powder

Both packed and unpacked beef masala powder were collected from local grocery shop. Two grams of beef masala powder was added into 50 ml of water and vigorously mixed. The quick settling indicates the presence of brick powder and slow settling indicates the absence of brick powder.

3.4.11. Detection of metanail yellow in beef masala powder

Two grams of beef masala powder was taken into a 10 mL water containing test tube and 2-3 drops of HCL added. Then instant violet color is appeared. Then the persistence of violet color when the solution diluted with water indicates the presence of metanail yellow in beef masala powder and the feebleness of violet color indicates the absence of metanail yellow.

3.4.12. Detection of Sudan Red III in fish masala powder

One gram of fish masala powder was transferred in a test tube and then 2 (two) ml of hexane added. Then shake it well and leave it to settle down. 2 ml of the acetonitrile reagent was added after decanting the clear solution into a different test tube and thoroughly shaking it. Appearance of a red color in the lower aceto-nitrile layer indicates Sudan red III is present and appearance of no red color indicates no Sudan red III is present.

3.4.13. Detection of metanail yellow in fish masala powder

Two grams of fish masala powder was taken into 10 mL of water (H_2O) in a test tube. And then 2-3 of HCL were added. Then instant violet color was appeared. Then the persistence of violet color when the solution diluted with water indicates the presence of metanail yellow in fish masala powder and the feebleness of violet color indicates the absence of metanail yellow.

Chapter 4: Result

4. Results

4.1. Microbial load in packed and un-packed species

The present work investigated the microbiological quality of food spicies (packed and un-packed) by the detection of total plate count (SPC), total coliforms (TC), *Salmonella*, *S. aureus* and mold count. Six types of spicies (total 120 samples, 60 packed and 60 un-packed) were evaluated that commonly available in markets and use for food preparation. In all spice samples (packed and unpacked) bacterial presence was detected with different ranges. In packed and unpacked spices, a minimum and highest range of total bacterial count was found in turmeric powders and the range is between 200 to 1,700 CFU (Table 4.1.1 and Table 4.1.2) and 380 to 1,660 CFU (Table 4.1.3 and Table 4.1.4) in each gram of sample. We did not observe the presence of total coliforms (fecal coliforms and *E. coli)* and *Salmonella* spp. in packed spices (Table 4.1.1 and Table 4.1.2) but, we found the presence of total coliform (TC) and *E. coli* in un-packed samples (Table 4.1.3 and Table 4.1.4).

Table 4.1.1 Microbiological quality assessment of six packed spices (total 60 samples, each packed spice contained 10 samples) commonly used in different food preparation (cooked foods) in Bangladesh.

Types of spices	SI	Parameters							Remark
		SPC (CFU/g)	TCC (CFU/g)	FC (CFU/g)	*E. coli* (CFU/g)	*Salmonella* spp. (CFU/g)	*Staphylococcus aureus* (CFU/g)	Mold (CFU/g)	
Beef masala	BM1	**1,140**	0	0	0	0	0	60	NCS
	BM2	**1,190**	0	0	0	0	0	20	NCS
	BM3	**1,230**	0	0	0	0	0	20	NCS
	BM4	800	0	0	0	0	0	30	CS
	BM5	400	0	0	0	0	0	30	CS
	BM6	**1,080**	0	0	0	0	0	50	NCS
	BM7	**1,110**	0	0	0	0	0	40	NCS
	BM8	**1,310**	0	0	0	0	0	70	NCS
	BM9	**1,160**	0	0	0	0	70	60	NCS
	BM10	**1,130**	0	0	0	0	30	70	NCSS
Chicken	CM1	**1,400**	0	0	0	0	25	0	NCS

masala	CM2	**1,120**	0	0	0	0	20	80	NCS
	CM3	**1,440**	0	0	0	0	0	**170**	NCS
	CM4	**1,350**	0	0	0	0	0	**170**	NCS
	CM5	**1,300**	0	0	0	0	21	20	NCS
	CM6	**1,400**	0	0	0	0	23	20	NCS
	CM7	**1,100**	0	0	0	0	0	20	NCS
	CM8	**1,200**	0	0	0	0	0	40	NCS
	CM9	**1,200**	0	0	0	0	0	20	NCS
	CM10	**1,100**	0	0	0	0	0	20	NCS
Fish curry masala	FCM1	**1,440**	0	0	0	0	15	**160**	NCS
	FCM2	**1,240**	0	0	0	0	12	**130**	NCS
	FCM3	**1,220**	0	0	0	0	12	**160**	NCS
	FCM4	**1,220**	0	0	0	0	26	30	NCS
	FCM5	**1,110**	0	0	0	0	15	20	NCS
	FCM6	**1,130**	0	0	0	0	0	20	NCS
	FCM7	850	0	0	0	0	0	20	CS
	FCM8	**1,300**	0	0	0	0	0	20	NCS
	FCM9	**1,400**	0	0	0	0	0	**110**	NCS
	FCM10	**1,470**	0	0	0	0	0	60	NCS
Chili powder	ChP1	600	0	0	0	0	0	30	CS
	ChP2	690	0	0	0	0	0	10	CS
	ChP3	410	0	0	0	0	0	80	CS
	ChP4	350	0	0	0	0	0	30	CS
	ChP5	330	0	0	0	0	22	30	CS
	ChP6	**1,100**	0	0	0	0	22	**120**	NCS
	ChP7	450	0	0	0	0	20	20	CS
	ChP8	390	0	0	0	0	20	40	CS
	ChP9	560	0	0	0	0	20	30	CS
	ChP10	**1,180**	0	0	0	0	11	**170**	NCS
Cumin powder	CuP1	**1,330**	0	0	0	0	0	70	NCS
	CuP2	**1,440**	0	0	0	0	0	70	NCS
	CuP3	**1,290**	0	0	0	0	12	100	NCS
	CuP4	**1,400**	0	0	0	0	0	100	NCS
	CuP5	600	0	0	0	0	0	50	CS
	CuP6	500	0	0	0	0	0	50	CS
	CuP7	**1,260**	0	0	0	0	0	80	NCS
	CuP8	**1,570**	0	0	0	0	0	80	NCS
	CuP9	**1,090**	0	0	0	0	0	60	NCS
	CuP10	**1,230**	0	0	0	0	0	**110**	NCS
Turmeric powder	TP1	200	0	0	0	0	0	**140**	NCS
	TP2	**1,050**	0	0	0	0	0	**120**	NCS
	TP3	**1,300**	0	0	0	0	22	**140**	NCS
	TP4	**1,700**	0	0	0	0	30	20	NCS
	TP5	710	0	0	0	0	0	50	CS
	TP6	800	0	0	0	0	0	70	CS
	TP7	800	0	0	0	0	0	10	CS
	TP8	600	0	0	0	0	0	10	CS
	TP9	800	0	0	0	0	0	30	CS
	TP10	550	0	0	0	0	0	60	CS
ISO		1,000	10/g			0/25g	100/g	100/g	

SI= Sample Identification, SPC= standard plate count, TCC= total coliforms count, FC= Fecal coliform, NCS= not conformed to standard, CS= conformed to standard, BM= beef masala, CM= chicken masala, FCM= fish curry masala, ChP= chili powder, CuP= Cumin powder, TP= turmeric powder, ISO= International Organization for Standardization, CFU= colony forming unit, g= gram, Remark indicates the total quality of products based on all parameters were evaluated in this study. Bold color indicates the value did not confirm the standard value of ISO.

Table 4.1.2: Statistical analysis predict the microbiological quality of packed spice samples.

Food Types	SPC	TCC	FC	*E. coli*	*Salmonella* spp.	*Staphylococcus aureus*	Mold
BM	1055 ± 265.72	0	0	0	0	10 ± 23.09	45 ± 19.58
CM	1261 ± 133.04	0	0	0	0	8.9 ± 11.56	56 ± 63.63
FM	1238 ± 183.77	0	0	0	0	8 ± 9.27	73 ± 60.56
CP	606 ± 304.38	0	0	0	0	11.5 ± 10.36	56 ± 51.68
CuP	1171 ± 352.4	0	0	0	0	1.2 ± 3.79	77 ± 21.11
TP	851 ± 417.28	0	0	0	0	5.2 ± 11.12	65 ± 51.48
p value	< 0.001	N/A	N/A	N/A	N/A	0.531	0.6854

BM = Beef masala, CM = Chicken masala, FM = Fish curry masala, CP = Chili powder, CuP = Cumin powder, TP = Turmeric powder. SPC= standard plate count, TCC= total coliforms count, FC= Fecal coliform, *E. coli* = *Escherichia coli*.

A minimum of 7 CFU (TC) and the highest 72 CFU (TC) was found in beef masala and turmeric powder. The highest 28 fecal coliforms were detected in fish curry masala and a minimum of 1 CFU was found in chili powder. *E. coli* was present in all un-packed spices with different ranges (minimum 4 CFU in beef masala and highest 49 CFU in turmeric powder in each gram of sample) (Table 4.1.3). Different levels of *Salmonella* spp. were present in un-packed spices (20% in beef masala, 30% in chicken masala, 50% in fish curry masala, 30% in chili powder, 40% in cumin and turmeric powder) (Table 4.1.5) but we did not detect the presence of *Salmonella* spp. in packed spices. *Staphylococcus aureus* was detected in packed (zero to 70 CFU per gram of sample) and un-packed spices (zero to 430 CFU per gram of sample). Different level of *S. aureus* was present in packed (20% in beef masala, 40% in chicken masala, 50% in fish curry masala, 60% in chili powder, 10% in cumin powder and 20% in turmeric powder) (Table 4.1.5) and un-packed (80% in beef masala, 100% in chicken masala, 70% in fish curry masala, 100% in chili powder, 50% in cumin powder.

Table 4.1.3. Microbiological quality assessment of six unpacked spices (total 60 samples, each unpacked spice contained 10 samples) commonly used in different food preparation (cooked foods) in Bangladesh.

Type of species	SI	Parameters							Remark
		SPC (CFU/g)	TCC (CFU/g)	FC (CFU/g)	*E. coli* (CFU/g)	*Salmonella* spp. (CFU/g)	*Staphylococcus aureus* (CFU/g)	Mold (CFU/g)	
Beef masala	BM1	**1,380**	**26**	15	11	0	20	**110**	NCS
	BM2	460	9	3	6	0	0	**120**	NCS
	BM3	890	7	3	4	0	0	**200**	NCS
	BM4	660	8	4	4	0	10	**130**	NCS
	BM5	**1,450**	**27**	14	13	2	10	**107**	NCS
	BM6	500	**11**	5	6	0	30	**150**	NCS
	BM7	**1,200**	**33**	17	16	1	20	**140**	NCS
	BM8	**1,100**	**23**	12	11	0	20	**170**	NCS
	BM9	**1,480**	**39**	26	13	0	20	**160**	NCS
	BM10	**1,080**	**27**	8	19	0	30	**170**	NCS
Chicken masala	CM1	**1,250**	**31**	11	20	2	**220**	**140**	NCS
	CM2	**1,440**	**23**	9	14	0	**120**	**180**	NCS
	CM3	**1,250**	**22**	7	15	0	**200**	**170**	NCS
	CM4	**1,180**	**37**	13	24	0	**300**	**170**	NCS
	CM5	**1,400**	**39**	18	21	0	**310**	**120**	NCS
	CM6	**1,510**	**45**	11	34	0	**430**	**120**	NCS
	CM7	**1,330**	**53**	21	32	1	**280**	**220**	NCS
	CM8	**1,290**	**36**	12	24	1	**230**	**140**	NCS
	CM9	**1,340**	**27**	8	19	0	**300**	**130**	NCS
	CM10	**1,360**	**44**	14	30	0	**140**	**120**	NCS
Fish curry masala	FCM1	**1,500**	**60**	28	32	0	**200**	**150**	NCS
	FCM2	**1,300**	**31**	6	25	2	**180**	**130**	NCS
	FCM3	**1,200**	**24**	9	15	1	**200**	**160**	NCS
	FCM4	**1,360**	**36**	13	23	1	0	**230**	NCS
	FCM5	**1,330**	**28**	12	16	1	**140**	**220**	NCS
	FCM6	**1,300**	**50**	17	33	0	**140**	**120**	NCS
	FCM7	750	**35**	12	23	0	**140**	**210**	NCS
	FCM8	900	**38**	11	27	0	**300**	**120**	NCS
	FCM9	**1,290**	**35**	10	25	0	0	**110**	NCS
	FCM10	**1,480**	**38**	12	26	1	0	**160**	NCS
Chili powder	ChP1	800	**17**	3	14	0	70	**130**	NCS
	ChP2	700	**12**	2	10	0	40	**108**	NCS
	ChP3	380	**14**	1	13	0	40	**180**	NCS
	ChP4	**1,630**	**48**	15	33	1	40	**230**	NCS
	ChP5	**1,660**	**63**	21	42	0	40	**130**	NCS
	ChP6	**1,650**	**54**	19	35	1	40	**120**	NCS
	ChP7	**1,330**	**63**	16	47	0	50	**120**	NCS
	ChP8	**1,330**	**51**	9	42	2	50	40	NCS
	ChP9	**1,400**	**53**	18	35	0	30	**130**	NCS
	ChP10	**1,450**	**46**	13	33	0	40	**170**	NCS
Cumin powder	CuP1	510	8	2	6	0	0	70	NCS
	CuP2	**1,300**	**20**	7	13	0	0	70	NCS
	CuP3	**1,220**	**41**	13	28	0	0	100	NCS
	CuP4	**1,110**	**51**	19	32	0	**130**	100	NCS
	CuP5	**1,240**	**62**	18	44	1	0	**150**	NCS
	CuP6	**1,210**	**65**	23	42	1	0	50	NCS
	CuP7	**1,330**	**50**	19	31	0	**110**	**180**	NCS
	CuP8	**1,100**	**33**	11	22	2	**120**	80	NCS
	CuP9	**1,100**	**26**	4	22	0	**130**	**160**	NCS
	CuP10	**1,430**	**60**	22	38	1	**160**	**110**	NCS

Turmeric powder	TP1	**1,200**	**29**	6	23	0	0	**140**	NCS
	TP2	**1,650**	**72**	23	49	0	0	**120**	NCS
	TP3	**1,200**	**48**	13	35	1	0	**140**	NCS
	TP4	**1,200**	**45**	12	33	1	**130**	**120**	NCS
	TP5	**1,210**	**26**	5	21	0	0	**150**	NCS
	TP6	**1,200**	**58**	13	45	0	0	**110**	NCS
	TP7	**1,380**	**31**	6	25	0	30	**210**	NCS
	TP8	**1,200**	**29**	2	27	1	0	**110**	NCS
	TP9	**1,100**	**28**	10	18	1	0	**230**	NCS
	TP10	**1,200**	**51**	12	39	0	0	**160**	NCS
ISO		1,000	10/g			0/25g	100/g	100/g	

SI= Sample Identification, SPC= standard plate count, TCC= total coliforms count, FC= Fecal coliform, NCS= not conformed to standard, CS= conformed to standard, BM= beef masala, CM= chicken masala, FCM= fish curry masala, ChP= chili powder, CuP= Cumin powder, TP= turmeric powder, ISO= International standards organization, CFU= colony forming unit, g= gram.

Remark indicates the total quality of products based on all parameters were evaluated in this study. Bold color indicates the value did not confirm the standard range of ISO.

And 20% in turmeric powder) spices (Table 4.1.5). The presence of mold in packed and unpacked spices was found from zero to 170 CFU and 40 to 230 CFU per gram of spices (Table 4.1.1,4.1.2, 4.1.3 and 4.1.4).

Table 4.1.4: Statistical analysis predict the microbiological quality of unpacked spice samples.

Food Types	SPC	TCC	FC	*E. coli*	*Salmonella* spp.	*Staphylococcus aureus*	Mold
BM	1020 ± 379.91	21 ± 11.44	10.7 ± 7.51	10.3 ± 5.16	0.3 ± 0.67	16 ± 10.75	145.7 ± 29.93
CM	1335 ± 98.35	35.7 ± 10.08	12.4 ± 4.38	23.3 ± 6.88	0.4 ± 0.7	253 ± 91.05	151 ± 33.15
FM	1241 ± 239.14	37.5 ± 10.5	13 ± 5.98	24.5 ± 5.82	0.6 ± 0.7	130 ± 101.21	161 ± 44.33
CP	1233 ± 448.06	42.1 ± 19.97	11.7 ± 7.47	30.4 ± 13.28	0.4 ± 0.7	44 ± 10.75	135.8 ± 50.05
CuP	1155 ± 250.74	41.6 ± 19.36	13.8 ± 7.55	27.8 ± 12.28	0.5 ± 0.71	65 ± 69.64	107 ± 43.22
TP	1254 ± 154.86	41.7 ± 15.61	10.2 ± 5.92	31.5 ± 10.47	0.4 ± 0.52	16 ± 41.15	149 ± 41.22
p-value	0.2399	0.0224	0.8217	< 0.001	0.944	< 0.001	0.0777

BM = Beef masala, CM = Chicken masala, FM = Fish curry masala, CP = Chili powder, CuP = Cumin powder, TP = Turmeric powder. SPC= standard plate count, TCC= total coliforms count, FC= Fecal coliform, *E. coli* = *Escherichia coli*.

Table 4.1.5. The percentages of prevalence (total coliforms, *Salmonella* spp., *Staphylococcus aureus* and Molds) in six packed and unpacked spices.

Packed spices				
	Total coliforms (%)	*Salmonella* spp (%)	*S. aureus* (%)	Molds (%)
Beef masala	0	0	0	0
Chicken masala	0	0	0	20
Fish curry masala	0	0	0	40
Chili powder	0	0	0	20
Cumin powder	0	0	0	10
Turmeric powder	0	0	0	30
Unpacked spices				
Beef masala	100	20	0	100
Chicken masala	100	30	100	100
Fish curry masala	100	50	70	100
Chili powder	100	30	0	90
Cumin powder	100	40	50	40
Turmeric powder	100	40	10	100

4.2. Detection of different adulterants in spices samples

Four different adulterants such as brick powder, Sudan red III, aniline dye and metanail yellow were evaluated in 120 spice samples (60 packed and 60 unpacked). Brick powder was present in unpacked spices such as beef masala and chicken masala powder (Table 4.2.1).

Sudan red III was detected in chili powder and fish curry powder. Aniline dye and metanail yellow dye were absent in all unpacked spices (Table 4.2.1).

Table 4.2.1: Detection of different adulterants in common unpacked food spices.

Unpacked spices	Brick powder	Sudan red III	Aniline dye	Metanail yellow
Chili powder	N/D	Present	N/D	N/D
Turmeric powder	N/D	N/D	N/D	N/D
Cumin powder	N/D	N/D	N/D	N/D
Beef masla powder	Present	N/D	N/D	N/D
Chicken masala powder	Present	N/D	N/D	N/D
Fish curry powder	N/D	Present	N/D	N/D

N/D (Not Detected)

Sudan red III and aniline dye were present in fish curry and turmeric powder. Brick powder and metanail yellow were absent in all types of packed spices (Table 4.2.2).

Table 4.2.2: Detection of different adulterants in common packed food spices.

Packed spices	Brick powder	Sudan red III	Aniline dye	Metanail yellow
Chili powder	N/D	N/D	N/D	N/D
Turmeric powder	N/D	N/D	Present	N/D
Cumin powder	N/D	N/D	N/D	N/D
Beef masala powder	N/D	N/D	N/D	N/D
Chicken masala powder	N/D	N/D	N/D	N/D
Fish curry powder	N/D	Present	N/D	N/D

N/D (Not Detected)

Chapter 5: Discussion

Minimization of food contamination, it is exceedingly important to confirm all steps are under ascetic conditions during food processing. In this regard, microbiological steps can play a crucial role to improve the quality of foods by the reduction of microbial contamination or ensure the presence of a maximum range by the following of ISO (International Organization for Standardization) guidelines. The present study was conducted to inspect the microbiological status (normal range of total bacterial count or harmful microorganism's) between packed and unpacked spices that are commonly used for food processing in Bangladesh. The spices producing company send a message to consumers (message generally labelled on the packet of spices) to pick their products due to health safety and our aim was to evaluate their statements. For these purposes, we have selected six type of packed spices (each type contained 10 spice samples which manufactured by well reported spices company, company name did not mention) and un-packed spices (prepared by the vendors). We investigate those which products (packed or un-packed spices) are much healthier for spice consumers.

Several organizations have indications for the limit of microorganism's or pathogens can present or be absent in food species in favor of human health. Among them, Bangladesh Standards and Testing Institute (BSTI), British Standards Institute (BSI), European Spice Association (ESA), Food and Drug Administration (FDA) and International Organization for Standardization (ISO) are well reported. However, specifications of microbial parameters in spices may be standardized in several countries with a compatible range of the organizations mentioned above. The results obtained from this study were compared with a range that was standardized by ISO.

Generally, the presence of Enterobacteriaceae used as a hygienic indicator of foods or spices that may cause serious illness to humans (Adams and Moss, 1995). The presence of fecal coliforms and *E. coli* in food or spices indicate that the products may be contaminated with fresh feces and the possible presence of enteric pathogens (Banerjee and Sarkar, 2003). In our study, we found that the packed species were free from contamination by total coliforms, *E. coli*, and *Salmonella* (Table 4.1.1). But all unpacked spices contained a high number of fecal coliforms and *E. coli* that crossed ISO limits and other standards limits (normal range- total coliforms <10 CFU g^{-1} and *Salmonella* spp. 0 (zero) CFU g^{-1} and a standard plate count of bacteria 1×10^{2} to 1×10^{3} CFU g-1) (Table 4.1.3). The percentage of spices contamination with *Salmonella* spp. was varied from sample to sample. In un-packed beef masala, 20% of samples were contaminated with *Salmonella* spp., 30% in chicken masala and chilli powder, 50% in fish curry masala, 40% in cumin and turmeric powder (Table 4.1.5). It is a reason of concern with these findings because most people of the country use unpacked spices to prepare their foods to lack hygienic knowledge. Besides, during the collection of non-packed spices vendors ensured that they did follow microbiological safety. Shamsuddeen (2009) reported on spice used for selling local meat (kilichi) in Northern Nigeria and the spice was highly contaminated with *E. coli* and *Salmonella* spp. Sagoo et al. (2009) studied spice and herbs in the United Kingdom (UK) found the presence of *E. coli* with a high range and crossed WHO limits that have potential health risks for spice consumers. Sospedra et al. (2010) reported that total coliforms were presented in dry spices with different ranges and failed WHO guidelines that were a threat for spice consumers. Most foodborne diseases are commonly caused by *Salmonella* spp. (Bakobie et al., 2017). We found a high number of total coliforms, *E. coli* and *Salmonella* spp. in un-packed spices that crossed ISO limits and our findings support their study (Table 4.1.3). In this study,

though we did not find the presence of total coliforms, *E. coli* and *Salmonella* in packed spices (Table 4.1.1), but most of the packed spices were crossed the limit of standard bacterial count (1,000 g^{-1}). In this sense, only 33.3% of packed spices were followed ISO limits and 66.7% of spices (Table 4.1.1) were not consumable to present a high number of total bacteria that crossed the ISO limits.

We observed that all un-packed samples were higher contaminated than packed spices with disease-causing pathogens. Un-packed spices such as beef masala and chili powders maintained by the acceptable ranges (100 CFU g^{-1}) were contaminated free from *S. aureus* but, other four un-packed spices were highly contaminated with *S. aureus* (chicken masala 100%, fish curry masala 70%, cumin powder 50% and turmeric powder 10%) (Table 4.3). Most of the packed spices were free from contamination with molds, but a high level of contamination was observed in all unpacked spices (100% contamination observed in beef masala, chicken masala, fish curry masala and turmeric powder, 90% in chili powder and 40% in cumin powder) (Table 4.3). In this case, the contaminated packed and unpacked spices are highly ricks for spice consumers. Man et al. (2016) reported that the contaminated spices commercialized in Romania may risk fungal and bacterial infection for spice consumers and we agree with their statement.

Both packed and unpacked spices were contaminated with different adulterants. But their percentage of contamination was higher in unpacked spices than the packed spices. Dhakal et al., 2016 reported that metanail yellow dye was found in turmeric powder that cause disease in human body. In our study we found different adulterants in spices samples such as Brick powder, Sudan dye, and Aniline dye that cause human diseases and we are agree with the findings of Dhakal et al., 2016.

The spices can be contaminated by many factors while manufactures and vendors prepared them. Liu et al. (2017) demonstrated that spices may be contained with microorganisms at various stages of preparation. Food handlers or equipment may have been associated with food contamination during processing (Moro et al., 2001). In this case of unpacked samples, vendors did not wear sterile hand gloves while they process spices. This may a great contamination source by which the spices can be contaminated. On the other hand, the unpacked spices are always uncovered while the vendors sell them, and the spice containers may not sterilize well. These exposes may increase the possible source of contamination by dust, atmospheric particles, and air-borne microbes. Bacteria proliferation from food production or processing to consumption is a process of chain that need to ensure good sanitary and handling practices in every stage (Amponsah-Doku et al., 2010; Pesewu et al., 2014). The spices produced by vendors and sold in local markets are not of good quality and a serious threat of health implication reported by Addo (2005) and we agree with their statements. This contamination may be possible to reduce by wearing sterile gloves in every stage during spice processing, handling, selling and sterilizing spice containers. In the case of packed spices, the manufacturers should increase their microbiological safety in every stage during spice processing that may help to reduce the possible contamination by total bacteria and molds.

Chapter 6: Conclusion

People have the right to consume safe and suitable food. Our study demonstrated that most of the packed spices (66.7%) were not consumable to cross the ISO limits of total bacterial. On the other hand, all unpacked spices were highly contaminated with pathogenic bacteria (fecal coliforms, *E. coli* or *Salmonella* spp.). *Salmonella* spp. and coliforms bacteria are responsible for the intestinal problem of humans. The presence of different adulterants in spice samples indicates that the spices are high risk for consumers health. The consumption of contaminated food spices may raise health problems such as diarrhea, cholera, stomach cramps, typhoid fever, or dysentery. The possible source of spice contamination includes storage equipment, handling, unhygienic environment, atmospheric particles, and airborne microbes. It is strongly recommended that periodic monetization of sanitary and certification of vendors by authority is essential to improve consistency.

Chapter 7: Recommendations and future perspectives

In the current study, unpacked spice quality was not so good due to microbial overload and some of adulterant's presence. And also, humidity was not maintained properly during the processing time. So, improvement during processing of the spices and maintaining required humidity may increase the quality of spices.

Some manual chemical laboratory test was performed in this study. But it would have been better to use perform modern qualitative and quantitative test like HPLC, FT-IR, FT-Raman, Paper Chromatography, MS (Mass Chromatography) etc. to have better and accurate result.

Overall acceptance of food and food products is usually influenced by its making method, ingredients and how they are processed. So, the modern instruments, standard procedure and quality control assessment should be ensured for consumer acceptance.

The results obtained from this study recommended that packed spices are more safety than unpacked spices. Unpacked spices are highly risk to the spice consumers to cross the ISO limit of microorganisms. To prepare the unpacked spices should follow all microbiological steps to minimize the bacterial contamination. In this case, government should increase their regular monitoring system in both cases such as packed and unpacked spices to minimize the microbial contamination, as well as the vendors locally produce the spices should take aware for different adulterants that can cause human diseases.

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Appendix A: Photo gallery

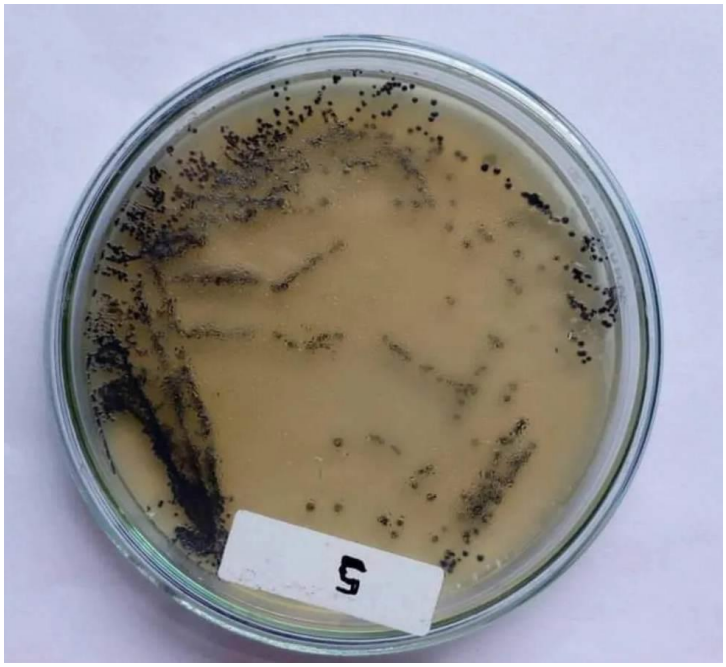

Fig: *Staphylococcus aureus* on BPA medium

Fig: *Salmonella*sp.OnXLD medium

Fig: Mold on PDA medium

Fig: Sample collection from grocery shop

Fig: Metanail yellow test in the spice of turmeric

Fig: Metanail yellow test in the spice of fish curry masala

Fig: Metanail yellow test in the spice of beef masala

Fig: Aniline dye test in the spice of turmeric powder

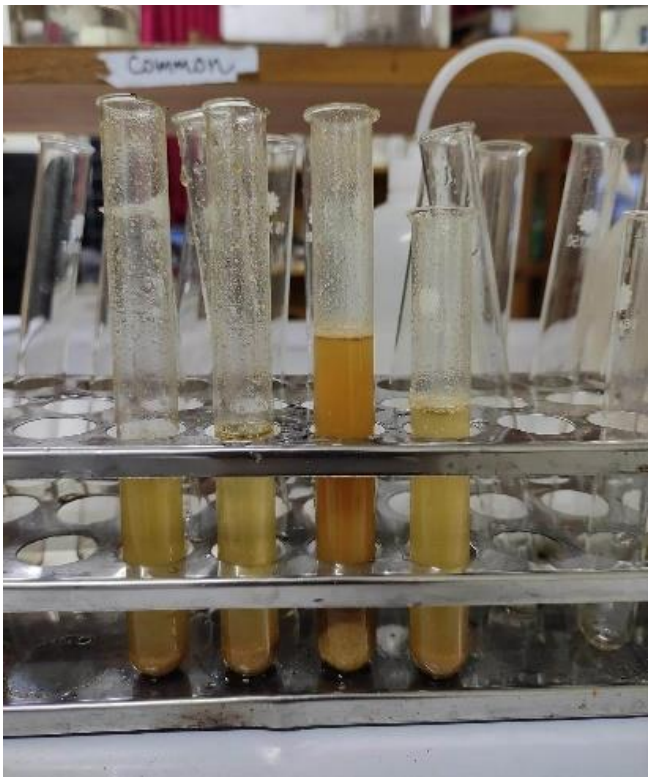

Fig: Determination of adulterants in cumin powder

Brief biography

Sabiha Sultana Mukta passed Secondary School Certificate (S.S.C) examination with Grade Point Average (GPA) 5.00 in 2011 from Dhamrai Hardinge High School, Dhamrai, Dhaka and Higher Secondary Certificate (H.S.C) with Grade Point Average (GPA) 5.00 in 2013 from Savar Cantonment Public School and College, Savar, Dhaka. Sabiha Sultana Mukta received the B.Sc. (Hon's) in Food Science and Technology From the faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh in 2018. Now she is a candidate for the degree of M.S. in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Technology, in Chattogram Veterinary and Animal Sciences University. She has boundless interest in exploration on the food safety and public health research in her area to improve the nutritional status of malnourished people in undeveloped and developing countries.