



# **Comparative Studies on Properties of Mango Seed Kernel Flour, Wheat Flour and Their Blends for Biscuit Production**

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**Department of Applied Chemistry & Chemical Technology**

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**Chittagong-4225, Bangladesh**

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**June, 2018**

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Session: January-June, 2016

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

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**JUNE 2018**



# *Dedication*

*I dedicate this small piece of work  
to my beloved parents*

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**The author**  
**June, 2018**

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## List of abbreviations

<b>Abbreviations</b>	<b>Elaboration</b>
<b>MKF</b>	Mango Kernel Flour
<b>gm</b>	Gram
<b>FAO</b>	Food and Agricultural Organization
<b>AAS</b>	Atomic Absorption Spectrophotometer
<b>AOAC</b>	Association of Official Analytical Chemists
<b>BBS</b>	Bangladesh Bureau of Statistics
<b>ml</b>	Milliliter
<b>0C</b>	Degree centigrade
<b>HCl</b>	Hydrochloric acid
<b>%</b>	Percentage
<b>0C</b>	Degree centigrade
<b>mg</b>	Milligram
<b>FAOSTAD</b>	Food and Agricultural Organization Statistics Database
<b>BGA</b>	Brilliant Green Agar
<b>BPA</b>	Buffered Peptone water
<b>H<sub>2</sub>O<sub>2</sub></b>	Hydrogen peroxide
<b>HNO<sub>3</sub></b>	Nitric acid
<b>Wt</b>	Weight
<b>kcal</b>	Kilo Calorie

## Abstract

After processing of ripe mango fruits, seeds and peels generate as waste which is 40-50% (approx.) of total fruit weight. The present study was carried out to produce mango seed kernel flour and to study its nutritional value. Then seed kernels flour was used to produce value added product. Physicochemical and functional properties of mango seed kernel flour, wheat flour and blend flour containing 10%, 20% and 30% mango seed kernel flour as substituting level for wheat flour were investigated. Biscuits prepared from wheat flour, and blend flour were subjected to proximate analysis and sensory evaluation. MKF was a good source of protein (6.25 g/100 g), fat (8.77 g/100 g), and energy (396.96 kcal/100 g). The 30% mango seed kernel flour analyses result showed higher bulk density (0.687 g/ml) and lower oil absorption (0.967 ml/g) ability among the blends. Biscuits containing 30% blend flour provided highest amount of fat (8.16 g/100 g), fiber (2.37 g/100 g) and energy (427.21 kcal) among the other blend flour biscuits. It also contained appreciable level of phosphorus (20.3 mg/100 g), calcium (69.17 mg/100 g) and iron (6.9 mg/100 g) among other sample which are important macro minerals required for vital functions of the body. Flour blends containing up to 30 % mango seed kernel flour were acceptable for biscuit production without adverse effect on sensory qualities. The 20% blend flour biscuits showed maximum value in color (7.85), flavor (8.42), taste (8.15) and overall acceptability (8.43) in sensory evaluation. The substitution of wheat flour with mango seed kernel flour can contribute as compatible resource for value added product manufacturing and waste utilization which might play significant role to food security in Bangladesh where mango fruit is abundant.

**Keywords:** *Mango seed kernel flour, Blend flour, Sensory characteristics, Biscuits, Chemical composition, Mineral content.*



## Chapter-1: Introduction

Wheat is one of the oldest of all cultivated plants. Today, there are more than 50000 cultivars of wheat in existence and as a result wheat can be grown in a relatively wide range of climatic conditions, especially in temperate climates (FAO, 1999). In addition to using wheat flour in making bread, wheat entered into other use and encouraged the growth and development of many industries such as biscuits, cakes, macaroni, pastas and others (Ahmed, 1995).

Wheat produced in Bangladesh does not meet the demand of consumption. For this reason more wheat is to be imported to supplement local production. In developing countries, looking for an alternative to wheat flour which cost them so much of their foreign currency. Some countries have successfully produced acceptable bread and biscuits from non-wheat flour.

Mango (*Mangifera indica* L.) is cultivated in tropical and subtropical parts of the world and is regarded as 'king of the fruit' in the Eastern due to its attractive color, delicious taste and exotic flavor. It is a rich source of carotenoids and provides high contents of ascorbic acid and phenolic compounds (Pott et al., 2003) that makes it the second most traded tropical fruit in the world and fifth in total production (FAOSTAD, 2015).

Mangoes are commercially cultivated in more than 103 countries worldwide and production is increasing each year due to increasing consumer demand. Approximately 77% of the world's mangoes are produced in Asian countries, while 13% and 9% are produced in the Americas and African countries, respectively (FAOSTAD, 2015). The Food and Agriculture Organization (FAO, 2007) estimate that the world's production of mango fruits is over 26 million tons annually.

The mango is indigenous to the Indian subcontinent and Southeast Asia (Fowomola, 2010). The world production of mango is estimated at 42 million tons per year; India is the largest producer of mango with 1,525,000 tons per year (FAOSTAD, 2015). Bangladesh hold's the 7<sup>th</sup> place among the 10<sup>th</sup> most producing countries as it produce about 1,047,850 tons of mango every year which accounts for 3.9% of the world's total mango production (FAO, 2014). According to the BBS, total mango production in 2015-2016 fiscal year is about 1,161,685tons. Different types of mangoes are grown in

Bangladesh such as- fazlee, gopalvog, lagda, foria, mishirvog, ashwina etc and 2<sup>nd</sup> most producing fruits (19.25%). Mangoes are mostly grown in Rajshahi and Chapainobabgonj districts.

As with many fruits, the edible fleshly portion or pulp of mango fruit is relished to the extent of commercialization. Mango pulp can be consumed at ripeness or in unripe, although most of the fruit is eaten fresh, and a wide variety of processed products can be prepared with the pulp (Ribeiro & Schieber, 2010). It can be canned, frozen as concentrates, mashed, dehydrated, minimally processed, or prepared as juices, nectar, jams, sauce, chutney and pickles (Masibo & He, 2009).

During the canning of fruits and vegetables and while preparing the juices, jams , jellies, dried products etc, large quantities of wastes materials are being left over. It has been reported that 45% of waste are from mangoes, 30% from citrus fruits and 10% from apples. High amounts of waste materials such as peels, seeds, stones etc. are resulted during the processing of fruits. The economic success of any food processing industry up to extend depends upon the utilization of waste products that are produced during the various stages of processing (Ribeiro & Schieber, 2010).

Botanically fruit peels and seeds are the most enriched part of fruits as they act as storage sites for nutrients required by young plant. Mango processing industries generate huge quantity of solid waste comprising mainly of peels and stones. Major components of mango fruit include pulp (45-60%), peel (20-30%) and stone (20-40%) (O'Shea et al., 2012); in the particular case of the seed, more than one million tons of mango seeds are annually produced as wastes, and these are not currently utilized for any commercial purposes (Leanpolchareanchai et al., 2014). In addition to the pulp of mangoes, mango seeds also contain valuable compounds, such as polyphenols, carotenoids, vitamins, and dietary fiber (DF) (Cristian et al., 2016).

Food wastes are biodegradable and create a problem to the processing industry, pollution monitoring agencies and the people who are concerned about its disposal. It is not only a problem, but a nuisance as well and therefore suitable methods have to be adopted to utilize them for conversion into value added products (Nand, 1998). Food by-products



represent a growing problem as the plant material is prone to microbial spoilage, which may cause odours and other environmental problems (Joshi & Attri, 2006; Laufenberg et al., 2003). For example, approximately \$10 million is spent annually on the disposal of only apple pomace in the USA (Shalini & Gupta, 2010). In another study, Schieber et al., (2001) reported that the costs to dry store and ship food by-products are economically limiting factors. Those fiber rich by products may be incorporated in food as inexpensive, non caloric bulking agents for partial replacement of flour, fat or sugar as enhancer of water and oil retention and to improve emulsion or oxidative stabilities.

The mango seed has aroused special scientific interest because it has been reported as a biowaste with high content of bioactive compounds (phenolic compounds, carotenoids, vitamin C, and dietary fibre) that improve human health (Jahurul et al., 2015). It is a good source of carbohydrates (58 to 80%) and protein (6 to 13%) and has an attractive profile of essential amino acids and lipids (6 to 16%); it is rich in oleic and stearic acids (Siaka, 2014). Additionally, the physicochemical characteristics of mango seed fat are very similar to those of commercial cocoa butter (Jahurul et al., 2015). The food value of mango seed kernel flour is reported to be nearly equal to that of rice and could be used in foods replacing cereal flours like rice, wheat, corn etc.

Therefore, in this research I investigated about the use of mango kernel as flour to utilize the byproduct and also investigated about MKF as a substitute of wheat flour to increases the nutritional value of baked food products at lower cost particularly in developing countries.

### **1.1 Objectives of the study**

In this context the present study was taken up with the following objectives:

- a) Utilization of mango seed kernel as mango kernel flour.
- b) Evaluation of physicochemical, microbiological, functional and nutritional properties of the mango kernel flour.
- c) Value addition of biscuit using mango seed kernel flour.
- d) Production of biscuit enriched with mineral at low cost.

## Chapter-2: Review of Literature

### 2.0 Origin and distribution of mango

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world and currently ranked 5th in total world production among the major fruit crops (FAO, 2008). It belongs to the genus *Mangifera* of the family Anacardiaceae. The genus *Mangifera* contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mangos belong to the species *Mangifera indica*. The other edible *Mangifera* species generally have lower quality fruit and are commonly referred to as wild mangos (Alcorne et al., 1999). According to some authorities, horticultural forms are derived from the species *M. indica*, considered indigenous to the eastern region of the Indian subcontinent, including Bangladesh, Assam (India), and Myanmar.

According to Alcorne et al., (1999) the genus *Mangifera* originated from tropical Asia, with the greatest number of species found in Borneo, Java, Sumatra, and the Malay Peninsula.

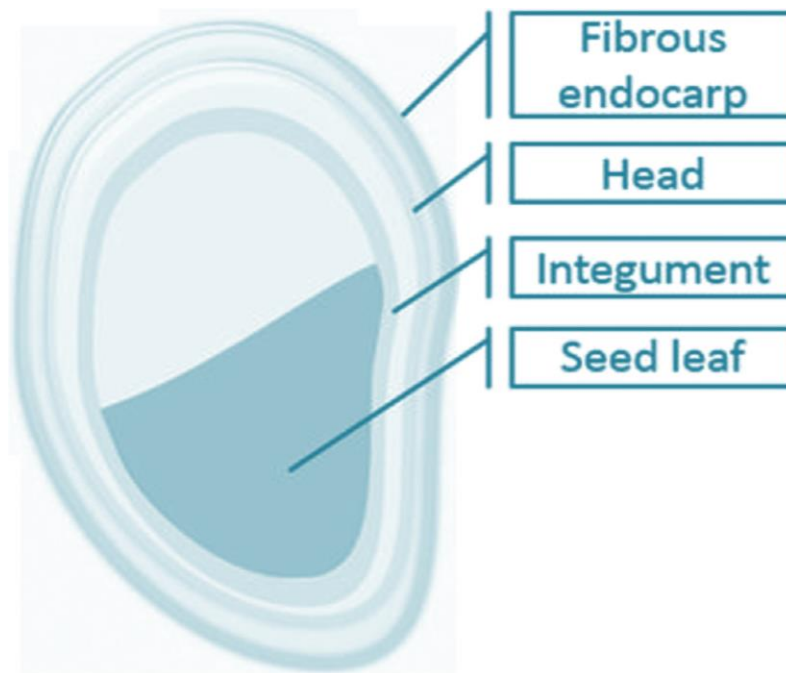
### 2.1. Description of Mango

#### Fruit

Mango fruit is classed as a drupe (fleshy with a single seed enclosed in a leathery endocarp). Fruits from different varieties can be highly variable in shape, color, taste, and flesh texture. Fruit shapes vary from round to ovate to oblong and long with variable lateral compression. Fruits can weigh from less than 50 g (0.35 lb) to over 2 kg (4.4lb). The fruit has a dark green background color when developing on the tree that turns lighter green to yellow as it ripens. Some varieties develop a red background colour at fruit set that remains until the fruits ripen. In addition to the background color, many varieties also have an orange, red, or burgundy blush that develops later in the fruit development, when the rind is exposed to direct sunlight. The mesocarp is the fleshy, edible part of the fruit that usually has a sweet and slightly turpentine flavor. When ripe, its colour varies from yellow to orange and its texture from smooth to fibrous (Fowomola, 2010).

## Seed

The mango fruit is classified as a deliquescent drupe; it contains a single seed surrounded by a fleshy mesocarp covered by a fibrous skin (Singh et al., 2013). As shown in Fig. 2.1, the seed is composed of a woody outer shell (endocarp) that is thick and hard and encloses a kernel (Masibo & He, 2009). The mango seed can be mono embryonic (produce a seedling) or poly embryonic (produce various plants); most of the varieties in India are mono embryonic, whereas the poly embryonic varieties are abundant in Myanmar, Thailand, Indonesia, and the Philippines (Tharanathan et al., 2006). Depending on the variety, the seed represents 10 to 25% of the total weight of the fruit, and the kernel represents 45 to 85% of the seed, or approximately 20% of whole fruit (Arogba, 1997).



**Figure 2.1: Internal and external structure of Mango seed.**

## **2.2 Waste generation by the processing industry**

Some parts of fruits are generally discarded as wastes whenever they are consumed. There is growing concern worldwide about the high number of byproducts created by the food industry, primarily including bagasse, peels, and seeds. In the majority of cases, byproducts represent greater mass (Ayala et al., 2011) and contain more active compounds than pulp or the end products (Morais et al., 2014).

According to Bhalerao and Mulmuley (1989), during fruit processing, more than 50% of fruit weight goes as waste in the form of peel, fruit stones etc. These wastes offer many opportunities for its direct conversion into by-products, which are essentially required by the industry as raw materials. Variety of products may be manufactured, if wastes are utilized in an organized manner.

El-Adawy and Taha (2001) reported that the problems of industrial wastes were becoming harder to solve, and much efforts would be needed to develop the nutritional and industrial potential of by-products, waste and underutilized agricultural products. Only a small portion of plant material is utilized directly for human consumption. The remainder, or part of it, may be converted into a nutrient for either food or feed or into fertilizer; thus an important contribution to food resources or industrial products could be made.

A large amount of waste poses the problem of its disposal without causing environmental pollution. Manufacture of useful by-products from this waste would not only result in reducing the cost of production of the main products but also solve the problem of waste disposal (Shrivastava and Kumar, 2006).

## **2.3 Waste generation by the mango processing industry**

During processing of mango, major by products such as peel and seed kernel are generated. The mango kernel contributed about 17-22% of the fruit (Soong and Barlow, 2006).

As seed kernel is not currently utilized for any commercial purposes, it is discarded as a waste and becoming a source of pollution. A preliminary study revealed that the seed represents from 20% to 60% of the whole fruit weight, depending on the mango variety

and the kernel inside the seed which represents from 45% to 75% of the whole seed (Maisuthisakul and Gordon, 2009).

According to Ahmad et al., (2007) after consumption or industrial processing of the fruits, considerable amounts of mango seeds are discarded as waste. According to mango varieties, the seed represents from 10% to 25 % of the whole fruit weight .The kernel inside the seed represents from 45% to75% of the seed and about 20% of the whole fruit. According to Tondon and Kalra (1989) mango peel and stones may be processed into value added products such as pectin, starch, oil and poultry feed.

#### **2.4 Technology for extraction of flour from seeds of Mango fruits**

Puravankara et al., (2000) reported that mango seed kernel powder (MSKP) from ripened mangoes of varieties such as Rajpuri, Langra, Kaiser, Alphonso and Desi were obtained by sun drying, deshelling, powdering the kernel and sieving through 100 mm sieve.

According to Arogba (1997) under-utilization of the whole mango kernel could be partly due to the limited knowledge of its toxicological properties.

Tannin was implicated as the bitter principle present in the kernel. Tannins are known to form a complex with protein and minerals, thereby reducing significantly the biological value of protein-rich foods (Millic, 1972; Rao and Prabhavathi, 1982).

The extent of complex formation depends on the relative concentration of the reactants (Millic, 1972). Furthermore, the toxicological effect of tannin depends on its composition (Millic and Stojanovic, 1972).

Arogba (1997) had reported that processing of mango stone involved cracking the shell of the seed manually, soaking the freed kernel in sulphited tap water (730 mg per liter) at  $28 \pm 2^\circ\text{C}$  for not more than 72 hours with occasional decantation and replacement with an equivalent amount of water until the water remained colorless.

Arogba (1999) had reported that mango kernel contained hydrolysable tannin which was about 75% of the total tannin content. These tannins would require treatment during processing in order to reduce their *in vivo* toxic effect. Furthermore, the rate of tannin formation through enzyme activity could be significantly reduced by water-blanching of mango kernel at  $90^\circ\text{C}$  for 5 min. The additional advantage of water-blanching is the possible leaching of soluble tannic substances into the soak-water.

Kaur et al., (2004) standardized the process of extraction of mango seed kernel flour. The mango stones were washed to remove any traces of adhering pulp and then dried at 40°C in a hot air cabinet drier for 10 hours. Kernels of each cultivar were removed from the stones after breaking them open, cut into small pieces (2 cm<sup>2</sup>) and steeped in water containing 0.16% sodium hydrogen sulphite for 12 hours at 50°C. The steep water was drained off, kernels were dried and the kernels were ground in a laboratory blender to get the powder.

Zein *et al.*, (2005) reported that soaking and boiling treatments had a great impact in reducing the anti-nutritional factors. The processed flour could be a principal ingredient for making products such as cakes and cookies for infants and adults and also other products such as bread and pastry.

## **2.5 Physicochemical, nutritional and functional properties of Mango seed kernel flour**

### **2.5.1 Physical properties**

According to Ashoush and Gadallah (2012) replacing 20, 30, 40 and 50% of wheat flour with MKF caused significant increase in biscuit diameter and thickness as compared with control. Dough development time and dough stability decreased when using MKF due to their high content of lipids.

Arogba (1997) studied the functional properties of MKF of Nigerian mango variety and reported that the kernel had water absorption capacity (190 %), Oil absorption capacity (86%) and bulk density (0.5 g / ml).

According to Legesse and Emire (2012) values of dispersibility for WF, MKF, MKWF<sub>1</sub>, MKWF<sub>2</sub>, and MKWF<sub>3</sub> were found 54.21%, 62.75%, 61.96%, 61.70% and 60.96%; respectively. The oil absorption capacities of each flour mix are WF (1.21 ml/g), MKF (0.83 ml/g), MKWF<sub>1</sub> (1.17 ml/g), MKWF<sub>2</sub> (1.14 ml/g), and MKWF<sub>3</sub> (1.11 ml/g).

Zazueta-Morales et al., (1999) evaluated the physicochemical and nutritional characteristics of mango kernel. Physical properties were reported as specific gravity

1.01; bulk density 580 kg/m<sup>3</sup>; water sorption index (WSI) 1.44 g water/g dry solids; water soluble solids (WSS) 12.14%; pH 4.5 and isoelectric point 5.0.

### 2.5.2 Chemical properties

Mango seed is fair in crude protein, high ether extract and low in fiber (Fowomola, 2010).

The table below shows the proximate composition of mango seed.

**Table 2.5: Proximate composition of mango seeds:**

Parameters	Composition (%)
Crude protein	10.06 ± 0.12
Crude oil	14.80 ± 0.13
Ash	2.62 ± 0.025
Crude fiber	2.40 ± 0.01
Carbohydrate	70.12 ± 1.34
Energy content	453.92 ± 4.32 KJ/100 g

Source: Fowomola (2010).

Fowomola (2010) reported that mango seed contained sodium (21.0 mg/100 g), potassium (22.3 mg/100 g), calcium (111.3 mg/100 g), magnesium (94.8 mg/100 g), iron (11.9 mg/100 g), zinc, (1.1 mg/100 g) and copper (0.1 mg/100 g) and also found that mango seed contained vitamin A 15.27 (IU), vitamin E (1.30 mg/100 g), Vitamin K (0.59 mg/100 g), Vitamin B1 (0.08 mg/100 g), Vitamin B2 (0.03 mg/100 g), Vitamin B6 (0.19 mg/100 g), Vitamin B12 (0.12 mg/100 g) and Vitamin C (0.56 mg/100 g).

Contents of fat, protein and ash in mango seed kernel ranged from 3.7-12.6%, 4-8.1% and 1-3.7% (on dry basis), respectively. Acid value of fat varied from 2.1 to 8.8 and unsaponifiables from 1 to 5.3% (Lakshminarayana et.al., 1983)

Elegbede et al., (1995) analysed mango seed kernel from Nigeria and found that it had 44.4% moisture, 6.0% protein, 12.8% fat, 32.8% carbohydrate, 2.0% crude fiber, 2.0%

ash and 0.39% tannin content. Mango kernel fat contained high amounts of stearic (46.3%) and oleic (40.0%), while palmitic, linoleic, arachidonic and behenic acids were present in small quantities (<6.6%). The inorganic ions detected, potassium (365 mg/100 g), phosphorus (140 mg/100 g), magnesium (100 mg/100 g) and calcium (49 mg/100 g) which were present in high levels, while iron, sodium, manganese and zinc were present in small levels (< 11.0 mg/100 g).

Ravindran and Sivakanesan (1996) evaluated the proximate composition, anti-nutrients, in-vitro protein digestibility and apparent metabolisable energy (AME) values of raw, water soaked and boiled mango seed kernel. Crude protein was 62.6 g/kg, 59g/kg and 57.6 g/kg respectively where as crude fat was estimated as 106 g/kg, 94 g/kg and 71 g/kg respectively. Crude fiber content was 19 g/kg, 22 g/kg and 27.5 g/kg while tannin content was 56.5 g/kg, 31.4 g/kg and 19 g/kg respectively in raw, water soaked and boiled mango seed kernels.

Depending on the variety, mango seed kernels contain on a dry weight basis 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash (Zein et al., 2005). Although mango seed kernels have a low content of protein, the quality of protein is good (Seleim et al., 1999).

Chemical composition of mango seed kernel was reported as lipids 6.83%; protein 7.93%, crude fiber 1.02%; ash 2.46%; carbohydrates 73.09%; *in-vitro* digestibility 69.19%; tannins 1.28 mg/g and phytic acid 1.73 mg/g; Protein profile observed were albumin, globulins (9.95%), prolamines, glutelins (73.55%) and non-protein nitrogen (18.16%). Starch was 70.76%, and main free sugars were glucose and fructose (0.1% each). Fatty acids profile (g/100 g oil) showed that the oil is rich in stearic (27.06%), oleic (45.39%) and linoleic (11.94%) acids. Significant amounts of calcium, copper and magnesium were found in the flour (Zazueta-Morales et al., 1999).

Bose et al., (2001) reported that mango seed kernel contained 7.5-8.8% fat, 6.1-6.8% protein, 1.3-2.4% crude fiber and 2.2-2.8% ash. The main fatty acids of the kernel fat are stearic (44.3-44.4%), oleic (38.9-42.1%), linolenic (4.5-7.4%) acid and palmitic (6.9-7.3%).



Zein et al., (2005) reported that mango seed kernels contained on an average 6.0% protein, 11% fat, 77% carbohydrate, 2.0% crude fiber and 2.0% ash on a dry weight basis.

Abdalla et al., (2007) reported that Egyptian mango seed kernels contained considerable amounts of total phenolic compounds and total lipids with high levels of unsaponifiable matter. Although mango seed kernels have a low content of protein, the quality of protein was good because it was rich in all essential amino acids. All the essential amino acids except methionine, threonine and tyrosin occurred at higher levels in the mango seed kernel than those of the FAO reference protein. Crude protein, total lipid, crude fiber and ash contents of mango seed kernel were 6.7%, 12.3%, 2.7%, and 2.5% respectively on a dry weight basis. Average moisture content of fresh mango seed samples were 50.7% and dried mango seed kernel powder samples were 8.5%.

## **2.6 Bioactive Compounds in MKF**

In the field of nutrition bioactive compounds are distinguished from essential nutrients. While nutrients are essential to the sustainability of a body, the bio active compounds are not essential since the body can function properly without them. These are natural compounds of foods that provide special health amenities (Biesalski et al., 2009).

The nutraceutical compounds present in fruits, such as antioxidant compounds, have aroused immense interest in the scientific community and from consumers due to beneficial effects against diseases such as cancer (Opie & Lecour, 2007).

Antioxidants of the peel and pulp include carotenoids, such as the provitamin A compound, beta carotene, lutein and alpha-carotene (Gouado et al., 2007).

Mahattanatawee et al., (2006) and Singh et al., (2013) reported that polyphenols such as quercetin, kaempferol, gallic acid, caffeic acid, catechins, tannins, and the unique mango xanthone, mangiferin are present in mango.

At present, almost all processed foods have synthetic antioxidants originating from the oil industry such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Carocho & Ferreira, 2013).

Consumption of synthetic antioxidants have harmful health effects (Lorenzo et al., 2013; Sarafian et al., 2002), which can be avoided by their substitution with natural antioxidants

such as those present in mango seed. The presence of phenolic compounds (mangiferin, isomangiferin, homomangiferin, quercetin, kaempferol, anthocyanins), phenolic acids (gallic, protocatechuic, ferulic, caffeic, coumaric, ellagic, 4-caffeoylquinic acids), and mineral antioxidants (potassium, copper, zinc, manganese, iron, selenium) is reported in mango seed (Ribeiro & Schieber, 2010).

Contents of these phytochemicals and nutrients appear to vary across different mango species (Rocha et al, 2007). Up to 25 different carotenoids have been isolated from mango pulp, the densest content for which was betacarotene accounting for the yelloworange pigmentation of most mango species (Chen et al., 2004). According to Barreto et al., (2008) peel and seed also have significant content of polyphenols, including xanthones, mangiferin and gallic acid.

## **Chapter-3: Materials and Methods**

The present investigation was conducted in the laboratory of the department of Food Processing and Engineering and Applied Chemistry and Quality Assurance laboratory in Chittagong Veterinary and Animal Sciences University (CVASU).

The study was carried out fewer than three experiments:

- Technology for extraction of mango seed kernel flour.
- Evaluation of physicochemical, functional, microbiological and mineral profile of mango seed kernel flour prepared from different cultivars of mango.
- Utilization of mango seed kernel flour for preparation of biscuits.

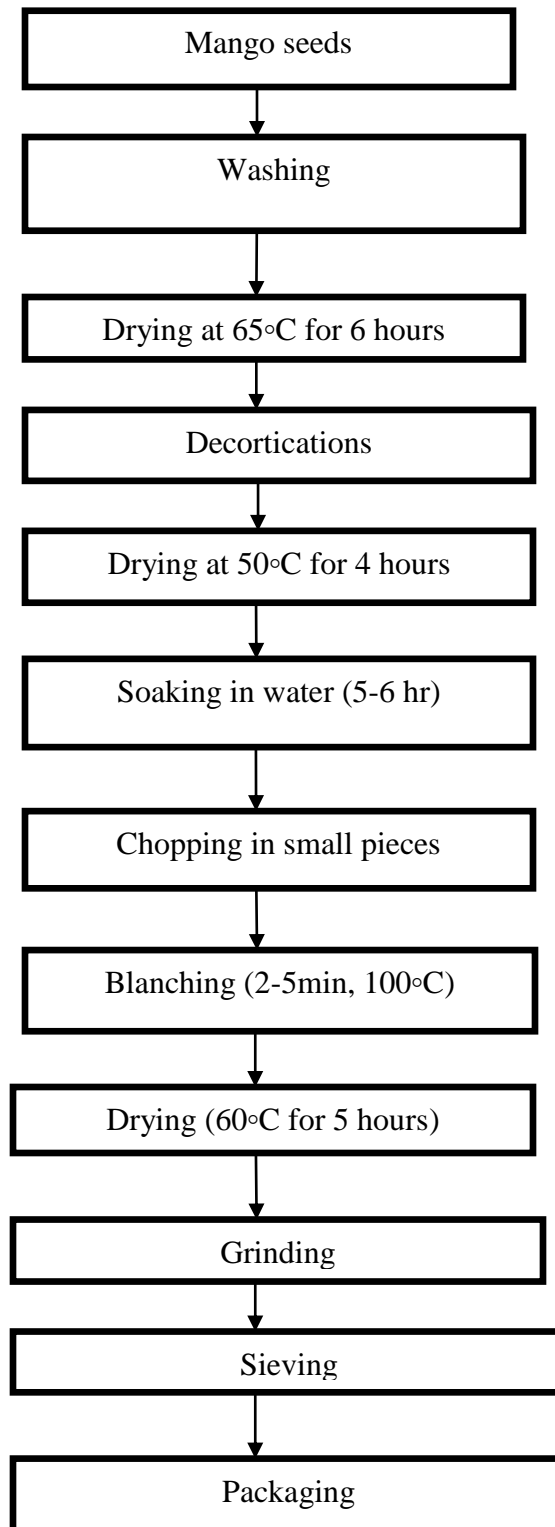
### **3.1. Materials**

The ripe mangoes of fazlee and ashwina varieties were procured from local market of Chittagong during the summer season of 2017. Commercial soft wheat flour, bakery fat, powdered sugar and skimmed milk were purchased from the local market. Food grade sodium chloride, ammonium bicarbonate and vanilla flavor were purchased from super shop in Chittagong for using biscuit processing.

### **3.2 Methods**

#### **3.2.1 Processing of mango seed into kernel flour**

Mango seeds were washed and dried in mechanical drier (Model: GENLAB 1000 Drying Cabinet) at 65°C for 6 hours. After drying, Kernels were separated from stone manually using stainless steel knife and dried in hot air oven at 50°C for 4 hours. During processing dried kernels were soaked (5-6 hours) in water, blanched (1-2 min), chopped into small pieces dried (60°C for 5 hours) and ground into flour in electric blender, sieved and stored in air tight container in 4°C (Yatnatti et al., 2014).



**Figure 3.2: Scheme of Production of Mango kernel Flour**

**Table 3.2: Recovery of Mango Kernel Flour (MKF)**

<b>Processing steps</b>	<b>Weight (g)</b>	<b>%increase in weight (%)</b>	<b>%decrease in weight (%)</b>
<b>Weight of kernels</b>	400	-	-
<b>Weight of kernels after soaking</b>	480	120	-
<b>Weight of kernel after drying</b>	342	-	85.5
<b>Weight of kernel after milling</b>	317	-	78
<b>Milling loss</b>	25	-	6.4
<b>Recovered flour after sieving</b>	301	-	75.25

### 3.2.2 Processing methods to reduce tannins in the mango seed kernels

Different processing methods are used for the reduction of tannin from mango seed kernel. Among them dehulling, soaking and blanching methods were used.

Dehulling is one of the physical treatments to remove the skin (coats) that contain unwanted substances such as tannins present in the skin. The skin, therefore, should be removed to reduce astringent taste. The soaking process involved slicing the pulp off the mango fruit, cracking the shell of the seed manually; grind the kernel, soaking the freed kernel in water. The ground de-skinned mango kernels were blanching in a mango kernel to water ratio of 1:2 (w/v) for 2-5 min at 100°C (Legesse and Emire, 2012).

### 3.3 Mango kernel and wheat flour blend formulation and biscuit processing:

Biscuits were prepared according to the modified method reported by Alkhalifa (1998). In table 3.3 blend of wheat and mango kernel flours for biscuit formulations are shown. Dough's containing 10, 20 and 30 % MKF as substituting levels for wheat flour were used for preparing biscuit samples according to the method described by Ashoush and Gadallah (2012). Biscuit dough was formulated by blending wheat flour, mango kernel flour with other ingredients according to Leelavathi and Haridas Rao (1993). The formulated blends were mixed for 15 min by manually. Each batches of the dough were removed from the mixing bowl and allowed to rest for 10 min. The dough pieces were sheeted and flattened using roller into a sheet of about 8 mm thickness, and then cut into different shapes. Samples were baked in an electric oven (DiFiore Forensic, Model, MLC80B, Forni morello, Italy, 1999) at 222°C for 25 min. After baking, biscuits were left to cool at room temperature and were stored in air tight container (Leelavathi and Haridas Rao 1993).

**Table 3.3: Formulation of dough containing mango kernel flour and other ingredients for biscuit development**

Ingredients	Biscuit dough formulation			
	Control	MKF <sub>1</sub>	MKF <sub>2</sub>	MKF <sub>3</sub>
Wheat flour (g)	500	450	400	350
MKF (g)	0	50	100	150
Butter (g)	300	300	300	300
Grind Sugar (g)	230	230	230	230
Milk powder (g)	3	3	3	3
Baking powder (NaHCO <sub>3</sub> )	5	5	5	5
Water (ml)	100	100	100	100
Salt (g)	2	2	2	2
Vanilla (ml)	2	2	2	2

Where: WF- wheat flour, MKF1- 10% mango kernel flour, MKF2 - 20% mango kernel flour and MKF3 -30% mango kernel flour as substituting levels for wheat flour.

### **3.4 Analysis methods:**

#### **3.4.1 Proximate chemical composition of MKF, Wheat flour and Biscuit:**

##### **3.4.1.1 Moisture content**

Moisture content was determined adopting AOAC (2005) method 14.004.

##### **Procedure**

About 5g sample was taken in a pre-weight crucible (provide with cover) which was previously heated to 130°C. The sample was dried for 1 hour in an air oven maintained at temperature 130°C. The crucible was while still in oven then transferred to desiccators and weighed immediately after reaching at room temperature. The loss of weight from sample was determined and the percent of moisture was calculated as follows:

$$\% \text{ Moisture content} = \frac{\text{Loss in weight}}{\text{weight of sample}} \times 100$$

Experiments were performed in three independent runs.

##### **3.4.1.2 Ash**

AOAC (2005) method 14.006 was used to determine the total ash content.

##### **Procedure**

A 5gm sample was weighed into clean, dry porcelain ashing dish which burned until white smoking stopped. The sample was then ignited with a gas burner until white smoking stopped. The sample was then placed in a muffle furnace at 550°C and ignited until light gray ash resulted (or to constant weight). The sample was then cooled in desiccators and weighed. The ash content was calculated by the following expression: Experiments were performed in three independent runs.

$$\% \text{ Ash} = \frac{\text{Loss in weight}}{\text{weight of sample}} \times 100$$

### 3.4.1.3 Crude Protein Content

Protein content was determined using AOAC (2005) method 2.049. The method was as follows:

#### Procedure

Crude Protein was estimated by using micro-kjeldahl method. Briefly, ten ml concentrated H<sub>2</sub>SO<sub>4</sub>, 0.2 g of sample and three g digestion mixture was taken in digestion tubes. Digestion system was switched on and the initial temperature of 100°C was set by pressing the temperature controller keys. The temperature controller was reset to 420°C. Mixture was heated till digested and proper water flow was regulated to ensure absolute removal of acid fumes. After digestion contents were cooled and distilled in classic-DX (VA). Distillation unit was switched on and green indication was ensured. The hose was connected to the steam release outlet and let it to the drain. Boric acid and alkali were filled in the bottles in required quantity. Sample to be digested was loaded and door was closed before switching on the power in control panel. System was ready for operation after receiving ready indication and the programme was selected by pressing 'run' key. Addition of boric acid and alkali was done. The distillate was then titrated with 0.1 N hydrochloric acid (HCl) to determine the ammonia absorbed in boric acid. Experiments were performed in three independent runs.

$$\text{Crude Protein} = \frac{14.01 \times (S - B) \times N \times 100}{W \times 1000}$$

Conversion factor of 6.25 was used to calculate percent protein.

$$\text{Crude Protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

Where,

N = Normality of acid used for titration (0.1 N HCl)

S = Volume of standard acid used for titration (ml)

B = Volume of 0.1 N HCl used for blank (ml)



#### 3.4.1.4 Crude Fat

AOAC (2005) method using Soxhlet apparatus was used to determine crude fat content of the samples.

##### **Procedure**

The dried sample was transferred to a thimble and plugged the top of the thimble with a wood of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet apparatus. Approximately 75ml or more of anhydrous petroleum ether was poured through the sample in the tube into the flask. Top of the fat extraction tube was attached to the condenser. The sample was extracted for 16 hours or longer on a water bath at 70-80°C.

At the end of extraction period, the thimble from the apparatus was removed and distilled of the petroleum ether by allowing it or collected in Soxhlet tube. When the petroleum had reached small, it was purer into a small, dry (previously weighed) beaker through a small funnel containing plug cotton. The flask was rinsed and filtered thoroughly using petroleum ether. The petroleum ether was evaporated on steam bath at low temperature and was then dried at 100°C for 1 hour, cooled and weighed. The difference in the weight gave the ether soluble materials present in the sample. The percent of crude fat was expressed as follows:

$$\% \text{ Crude fat} = \frac{\text{Weight of ether soluble material}}{\text{weight of sample taken}} \times 100$$

#### 3.4.1.5 Crude Fiber Content

Crude fiber was estimated by standard AOAC method of analysis (AOAC, 2000).

##### **Procedure**

One g of fat free dried sample was weighed and put in one litre tall beaker and 200 ml of 1.25% H<sub>2</sub>SO<sub>4</sub> was added. The solution was kept boiling for 30 minutes under bulb condensers. Beaker was rotated occasionally to mix the content and removed the particles from the sides. Content of the beaker was filtered through funnel. Sample was washed

back into tall beaker with 200 ml, 1.25 per cent sodium hydroxide, brought to boiling point and boiled exactly for 30 minutes. All insoluble matter was transferred to the sintered crucible by means of boiling water until it became acid free, washed twice with alcohol, three times with acetone, dried at 100°C to constant weight, reweighed and ashed in a muffle furnace at 550 °C for 1 hr. Crucible was cooled in a dessicator, reweighed and percentage of crude fiber in the samples was calculated.

$$\text{Crude fiber} = \frac{(W_2 - W_3) \times 100}{W_1}$$

Where,

W1 = Weight (g) of Sample (bun)

W2 = Weight (g) of insoluble matter

W3 = Weight (g) of ash

#### **3.4.1.6 Total carbohydrate**

Total carbohydrate content of the sample was determined as total carbohydrate by difference, that is by subtracting the measured protein, fat, ash and moisture from 100 (Pearson, 1970).

#### **3.4.1.7 Energy content**

The energy content of the baby food samples was determined by calculating the amount of protein, fat and carbohydrate of respective food items and by using the following equation (Parvin et al., 2014).

$$\text{Energy} = (\text{Protein} \times 4.1) + (\text{Fat} \times 9.3) + (\text{Carbohydrate} \times 4.1)$$

### 3.4.2 Functional properties of flour

Mango kernel and wheat flour and their blends were tested for functional properties such as bulk density, water holding capacity, water absorption capacity, oil absorption capacity, and hydrophilic-lipophilic index.

#### 3.4.2.1 Bulk density

Bulk density of sample was determined by method of Okala and Potter (1977). 10 g of flour was put into a 100 mL measuring cylinder and tapped to constant volume. The bulk density ( g cm<sup>-3</sup>) calculated using the formula:

$$\text{Bulk density} = \frac{\text{weight of flour(g)}}{\text{flour volume(cm}^3\text{)}}$$

#### 3.4.2.2 Water retention capacity

Water holding capacity was determined by a modified method of Gould et al., (1989). One gram of the flour sample was weighed into centrifuge tube, 10mL distilled water was added and vortexed for 30 sec. The sample was allowed to hydrate for 30 min at 300 rpm using a bench top centrifuge. The supernatant was discarded and the hydrate sample was weighed. Then the sample was dried at 105°C to constant weight. Experiments were done in three independent runs.

$$WRC \left( \frac{g \text{ water}}{g \text{ dry sample}} \right) = \frac{P2 - P1}{P0}$$

Where,

P<sub>0</sub> (g) = Weight of dry sample.

P<sub>1</sub> (g) = Final constant weight of dried sample.

P<sub>2</sub> (g) = Weight of wetted residue.

### 3.4.2.3 Oil Retention Capacity (ORC)

The oil retention capacity (ORC), also known as fat absorption capacity, was determined as described elsewhere (Sosulski, 1962; Raghavendra et al., 2006). Briefly, 1 g of dry flour sample was put in centrifuge tube of known total weight to which 10 ml of refined oil were added and allowed to equilibrate overnight. It was then centrifuged at 3000g for 20 min. The supernatant was removed and the weight of oily residue plus centrifuge tube was recorded.

$$ORC \left( \frac{\text{g of oil absorbed}}{\text{g of dry sample}} \right) = \frac{P2 - P1}{P0}$$

Where,

P0 (g) = Weight of dry sample;

P1 (g) = Weight of dry sample + Centrifuge tube;

P2 (g) = Weight of oily residue + Centrifuge tube.

### 3.4.2.4 Determination of pH of Samples:

The pH of samples was measured by the standard AOAC method using a pH meter (AOAC, 2003). Briefly, 10g of flour samples were suspended in 75 ml of distilled water and allowed to macerate for 30 min. The suspension was filtered and the pH of the dispersion obtained was measured. Experiments were done in three independent runs.

### 3.4.3 Physical measurements of biscuits

The spread factor, width and thickness of biscuits were evaluated according to AACC (2000), method no. 10-50D. The spread ratio was calculated by dividing (W) by thickness (T).

### 3.4.4 Sensory evaluation of biscuits

Biscuits incorporated with mango kernel flour and controls were coded with different numbers. All samples submitted to sensory evaluation by a panel of ten member including teacher, student, and production officer of Banoful & Co. The panelists were

asked to rate each sensory attribute using the control biscuits as the basic for evaluation. Biscuits were evaluated for surface color, texture, taste, flavor and overall quality on a 9-point hedonic scale, where 9 indicated 'like extremely' and 1 'dislike extremely'.

### **3.4.5 Bacteriological isolation and identification**

The bacteriological investigation of the samples was done in the Poultry Research and Training Centre (PRTC) laboratory in Chittagong Veterinary and Animal Sciences University.

#### **3.4.5.1 Isolation of *Escherichia coli***

Pre-enrichment of *E. coli* were done in BPA broth (Oxoid ltd, Basingstoke, Hampshire, UK) of the samples (Thaker et al., 2013). A loopful of culture inoculates on MacConkey (Oxoid ltd, Basingstoke, Hampshire, UK) agar. Pink colonies obtained from MacConkey agar were taken and inoculated on Eosin methelene blue (EMB) (Oxoid ltd, Basingstoke, Hampshire, UK) agar to verify whether the bacterial population was *E. coli*, or not. Dyes Eosin and Methylene Blue react with products released by *E. coli* from lactose or sucrose as carbon and energy source, forming metallic green sheen regarded as positive isolate (Virpari et al., 2013).

#### **3.4.5.2 Isolation of *Salmonella sp.***

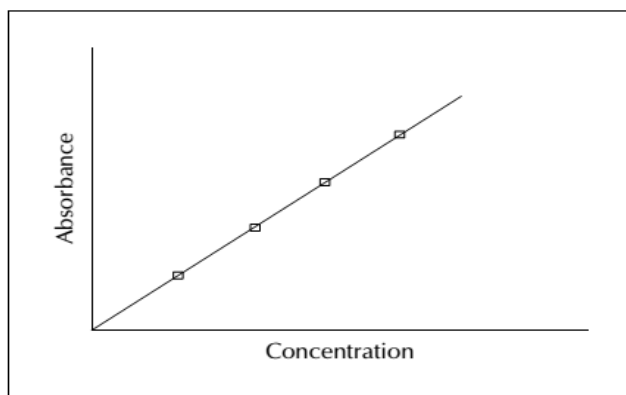
The samples were pre-enriched in BPA (Oxoid ltd, Basingstoke, Hampshire, UK), incubated at 37°C for 16 hours. One ml of inoculums was transferred into Selenitecystein broth (Oxoid ltd, Basingstoke, Hampshire, UK) after pre-enrichment (Carrique-Mas and Davies, 2008). A loopful of inoculums plated onto Xylose Lysine Deoxycholate (XLD) (Oxoid ltd, Basingstoke, Hampshire, UK) medium and incubated at 37°C for 24 hrs. Black centered colony from XLD was inoculated in Brilliant Green Agar (BGA) (Oxoid ltd, Basingstoke, Hampshire, UK) and incubated as well.

### **3.4.6 Analysis of minerals**

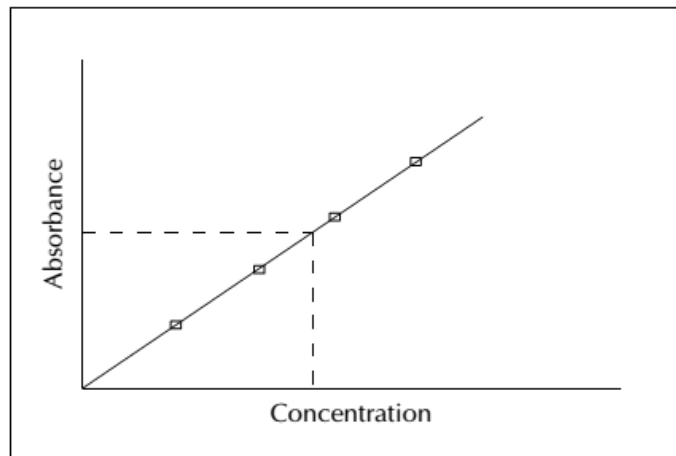
The contents of P, Ca, and Fe were measured after digestion in HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> by atomic absorption spectrophotometer (Shimadzu model AA-7000) similar to a study carried out by AOAC (2016).

## Procedure

All samples were dried in drying oven at 105°C to a constant weight. After drying 0.2 g samples were weighed into digestion vessels. Then 5 mL HNO<sub>3</sub> and 2 mL 30% H<sub>2</sub>O<sub>2</sub> were added. Vessels were then closed and placed in holder. Vessel holder then placed in microwave oven and exposed to defined program parameters 250 watts for 3 min, 630 watts for 5 min, 500 watts for 22 min and final 0 watts for 15 min. Then removed digestion vessels from microwave oven and cooled thoroughly before opening them. Vessel were then opened and rinsed down lid and walls into container. The solutions were then transferred to 25 mL volumetric flask and dilute to mark with deionized water. Then solution transferred to plastic container. For blank, same procedure was carried out. Further dilution for test solution was done with 3M HNO<sub>3</sub>. The concentration of Fe, P and Ca were determined by Flame techniques in AAS. A calibration curve is used to determine the unknown concentration of an element. The instrument is calibrated using several solutions of known concentrations. A calibration curve is produced which is continually rescaled as more concentrated solutions are used – the more concentrated solutions absorb more radiation up to a certain absorbance. The calibration curve shows the concentration against the amount of radiation absorbed. Calibration curves were plotted for each of the metal standard solution.



When sample solution fed into the instrument and the unknown concentration of the element then displayed on the calibration curve



**Figure 3.4.6: Calibration curve of for determination of minerals (by AAS)**

### **3.5 Statistical analysis**

The obtained data were stored in Microsoft Excel 2013 and then significant differences were determined by one-way analysis of variance (ANOVA) and Duncan's multiple range test using R Statistical Software (version 3.4.1; R Foundation for Statistical Computing, Vienna, Austria). The significance level was set at the level of  $p < 0.05$ .

## Chapter-4: Results

The results of the study are presented under the following major headings in this chapter.

### 4.1 Proximate composition of Mango kernel flour, wheat flour and blend flour:

The chemical and nutritional constituents of mango seed kernel flour recorded were moisture, ash, fat, protein, carbohydrate, fiber which are shown in Table-4.1.

The moisture content, crude protein, crude fat, total ash, total carbohydrate and crude fiber of raw mango kernel flour were 12.64%, 5.25%, 8.77%, 1.79%, 70.92%, and 3.36% respectively. Crude protein content of the blend flour ranged from 9.57% to 10.38%. Crude fat, ash, fiber and carbohydrate content of MKF, wheat flour and blend flour varied from 1.45% to 8.77%, 0.86 to 1.79%, 0.79% to 3.36%, and 70.92% to 74.33% respectively.

**Table 4.1: Proximate composition and calorific value of wheat flour, MKF and blended flours (g/100g DM):**

Flour type	Parameters					Total	Energy
	Protein	Fat	Ash	Fiber	Moisture	Carbohydrate	
<b>Control (Wheat flour)</b>	11.34 ± 0.121 <sup>a</sup>	1.45 ± 0.035 <sup>b</sup>	0.86 ± 0.089 <sup>b</sup>	0.79 ± 0.040 <sup>b</sup>	12.0 ± 0.075 <sup>b</sup>	74.21 ± 0.83 <sup>a</sup>	363.77 ± 1.56 <sup>c</sup>
<b>MKF<sub>1</sub></b>	10.38 ± 0.153 <sup>a</sup>	2.40 ± 0.032 <sup>b</sup>	1.01 ± 0.036 <sup>b</sup>	0.93 ± 0.036 <sup>b</sup>	11.87 ± 0.090 <sup>b</sup>	74.33 ± 0.175 <sup>a</sup>	369.69 ± 0.46 <sup>bc</sup>
<b>MKF<sub>2</sub></b>	9.96 ± 0.065 <sup>a</sup>	2.98 ± 0.035 <sup>b</sup>	1.03 ± 0.049 <sup>b</sup>	1.08 ± 0.025 <sup>b</sup>	11.88 ± 0.025 <sup>b</sup>	73.85 ± 0.735 <sup>a</sup>	371.12 ± 2.51 <sup>bc</sup>
<b>MKF<sub>3</sub></b>	9.57 ± 0.065 <sup>a</sup>	3.80 ± 0.055 <sup>b</sup>	1.13 ± 0.026 <sup>b</sup>	1.22 ± 0.015 <sup>b</sup>	11.75 ± 0.03 <sup>b</sup>	73.7 ± 0.153 <sup>a</sup>	376.96 ± 0.12 <sup>b</sup>
<b>MKF</b>	6.25 ± 0.075 <sup>b</sup>	8.77 ± 0.130 <sup>a</sup>	1.79 ± 0.031 <sup>a</sup>	3.26 ± 0.065 <sup>a</sup>	12.64 ± 0.145 <sup>a</sup>	69.92 ± 0.38 <sup>b</sup>	396.96 ± 0.058 <sup>a</sup>

All values are means ± SD. DM, each values are expressed on dry matter basis



a-c Means not sharing a common superscript letter with in a column are significantly different ( $P < 0.05$ )

Where, Control = wheat flour, MKF= mango kernel flour, MKWF<sub>1</sub>=10% MKF and 90% WF, MKWF<sub>2</sub>= 20% MKF and 80% WF and MKWF<sub>3</sub>= 30% MKF and 70%

#### 4.2 Evaluation of physical and functional properties of mango kernel and blend flour:

The physical and functional qualities of wheat flour and kernel flour obtained from mango were analyzed and data presented in Table-4.2

The bulk density value of wheat flour, mango kernel flour and blend flour are shown in table 4.2. It ranged from 0.51-0.76 g/ml. The bulk density was higher for MKF which is 0.767 g/ml and lower for wheat flour which is 0.573 g/ml.

The values of oil absorption for WF, MKF, MKF<sub>1</sub>, MKF<sub>2</sub>, and MKF<sub>3</sub> were 1.10 ml/g, 0.87 ml/g, 1.07 ml/g, 1.0 ml/g, and 0.97ml/g respectively.

The value of water absorption of wheat flour was 3.45% and for MKF is 3.9%. The blending of MKF with WF significantly increases water absorption capacity.

pH values of control, MKF and blends flour are ranged from 6.17 to 5.55.

**Table 4.2: Functional and physical properties of mango kernel flour, wheat flour and blend flours:**

Flour type	Bulk density (g/ml)	Oil absorption (ml/g)	Water absorption (w/w, %)	pH
Control(WF)	0.517±0.0153 <sup>e</sup>	1.107±0.0115 <sup>a</sup>	3.457±0.0208 <sup>e</sup>	6.177±0.021 <sup>a</sup>
MKF	0.767±0.0153 <sup>a</sup>	0.867±0.0208 <sup>d</sup>	3.923±0.02517 <sup>a</sup>	5.55±0.02517 <sup>c</sup>
MKF <sub>1</sub>	0.573±0.0153 <sup>d</sup>	1.07±0.02 <sup>a</sup>	3.563±0.02517 <sup>d</sup>	5.96±0.03 <sup>a</sup>
MKF <sub>2</sub>	0.653±0.0153 <sup>c</sup>	1.007±0.0115 <sup>b</sup>	3.647±0.01528 <sup>c</sup>	5.9±0.0351 <sup>b</sup>
MKF <sub>3</sub>	0.687±0.0058 <sup>b</sup>	0.967±0.0058 <sup>c</sup>	3.737±0.02309 <sup>b</sup>	5.653±0.01 <sup>b</sup>

All values are means ± SD.

Where, Control = wheat flour, MKF = mango kernel flour, MKWF<sub>1</sub> =10% MKF and 90% WF, MKWF<sub>2</sub> = 20% MKF and 80% WF and MKWF<sub>3</sub> = 30% MKF and 70% WF

a-e Means not sharing a common superscript letter with in a column are significantly different ( $P < 0.05$ )

### 4.3 Proximate composition of biscuits:

The proximate analysis of biscuits containing wheat flour and mango kernel flour with different blend ratios is shown in Table-4.3.

The crude protein, moisture content, crude fat, total ash, total carbohydrate and crude fiber of control (wheat flour) were 7.5%, 4.88%, 6.88%, 0.87%, 79.88% and 0.98%. Protein content varied from 6.16 to 5.5% and fat content varied from 7.25 to 8.16% among the biscuits prepared with blend flour.

**Table 4.3: Proximate composition (g/100g DM), and calorific value of biscuits containing mango kernel flours:**

Biscuit type	Parameters						
	Protein	Fat	Ash	Fiber	Moisture	Carbohydrate	Energy (kcal)
Control (WF)	7.5 ± 0.015 <sup>a</sup>	6.88 ± 0.02 <sup>d</sup>	0.87 ± 0.005 <sup>d</sup>	0.98 ± 0.005 <sup>c</sup>	4.88 ± 0.015 <sup>d</sup>	79.88 ± 0.036 <sup>b</sup>	422.11 ± 0.17 <sup>c</sup>
MKF <sub>1</sub>	6.16 ± 0.031 <sup>bc</sup>	7.25 ± 0.015 <sup>c</sup>	0.91 ± 0.01 <sup>c</sup>	1.74 ± 0.021 <sup>b</sup>	4.92 ± 0.025 <sup>c</sup>	80.76 ± 0.045 <sup>a</sup>	423.80 ± 0.076 <sup>b</sup>
MKF <sub>2</sub>	6.45 ± 0.026 <sup>b</sup>	7.87 ± 0.015 <sup>b</sup>	0.97 ± 0.017 <sup>b</sup>	1.38 ± 0.015 <sup>bc</sup>	5.0 ± 0.005 <sup>b</sup>	79.07 ± 0.051 <sup>b</sup>	426.0 ± 0.011 <sup>a</sup>
MKF <sub>3</sub>	5.55 ± 0.047 <sup>c</sup>	8.16 ± 0.035 <sup>a</sup>	1.06 ± 0.01 <sup>a</sup>	2.37 ± 0.02 <sup>a</sup>	5.08 ± 0.005 <sup>a</sup>	80.14 ± 0.040 <sup>ab</sup>	427.21 ± 0.120 <sup>a</sup>

All values are means ± SD. DM, each values are expressed on dry matter basis

a-d Means not sharing a common superscript letter with in a column are significantly different ( $P < 0.05$ )

### 4.4 Physical measurements of biscuits:

The data of physical measurement of biscuits are shown in Table-4.4. The spread ratio, width and thickness values of control, MKF<sub>1</sub>, MKF<sub>2</sub>, and MKF<sub>3</sub> biscuits sample were ranged from 96.51 to 86.10, 60.41 to 68.01, and 7.9 to 6.26; respectively.

**Table 4.4: Physical measurement of biscuits containing mango kernel flours:**

Types of biscuit	Parameter		
	Width ,w(mm)	Thickness ,T (mm)	Spread ratio(W/T)
Control	60.41±0.005 <sup>c</sup>	6.26±0.02 <sup>d</sup>	96.51±0.31 <sup>a</sup>
MKF <sub>1</sub>	64.9±0.01 <sup>b</sup>	6.84±0.015 <sup>c</sup>	94.93±0.22 <sup>b</sup>
MKF <sub>2</sub>	66.28±0.01 <sup>b</sup>	7.29±0.021 <sup>b</sup>	90.84±0.27 <sup>b</sup>
MKF <sub>3</sub>	68.01±0.036 <sup>a</sup>	7.90±0.015 <sup>a</sup>	86.05±0.12 <sup>c</sup>

All values are means ± SD. a-d Means not sharing a common superscript letter with in a column are significantly different (P < 0.05).

#### 4.5 Microbiological investigation of biscuits:

Microbial population was enumerated in the samples. There were no growth of *Escherichia coli*, and *Salmonella sp.* of any sample. This data are given in table 4.5.

**Table 4.5: Microbiological analysis of biscuits containing mango kernel flours:**

Types of biscuit	<i>Escherichia coli</i>	<i>Salmonella sp</i>
Control	0	0
MKF <sub>1</sub>	0	0
MKF <sub>2</sub>	0	0
MKF <sub>3</sub>	0	0

#### 4.6 Minerals content of biscuits:

Mineral content of the sample are shown in Table-4.6. The amount of P, Ca and Fe of control, MKF<sub>1</sub>, MKF<sub>2</sub>, and MKF<sub>3</sub> biscuits sample were ranged from 245.33 to 281 mg, 53.12 to 48.8 mg and 5.6 to 4.7 mg; respectively.

**Table 4.6: Minerals content of Control and biscuit containing blend flour**

Sample	Minerals(mg/100g)		
	P	Ca	Fe
Control	51±0.04 <sup>a</sup>	48.8±0.2 <sup>d</sup>	4.7±0.12 <sup>d</sup>
MKF <sub>1</sub>	45.33±0.15 <sup>b</sup>	53.12±0.025 <sup>c</sup>	5.6±0.25 <sup>c</sup>
MKF <sub>2</sub>	34.67±0.15 <sup>b</sup>	61.17±0.153 <sup>b</sup>	6.1±0.04 <sup>b</sup>
MKF <sub>3</sub>	20.3±0.53 <sup>c</sup>	69.17±0.208 <sup>a</sup>	6.9±0.03 <sup>a</sup>

All values are means ± SD.

a-d Means not sharing a common superscript letter with in a column are significantly different (P < 0.05)

#### 4.7 Sensory qualities of biscuits:

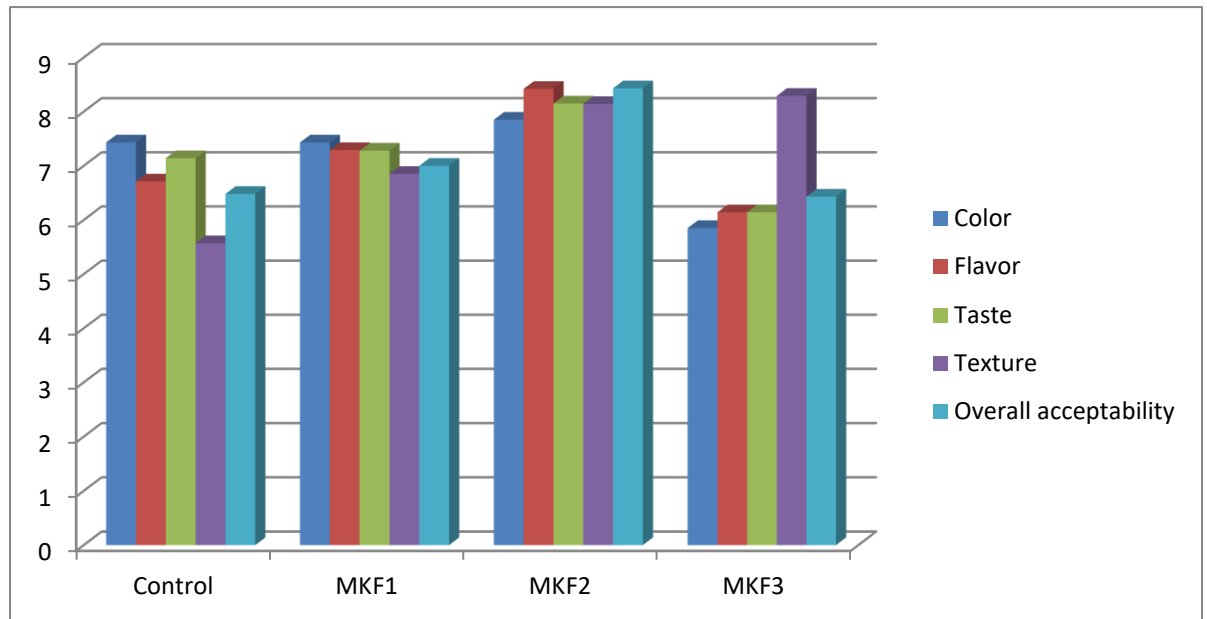
Results of sensory attributes in terms of color, flavor, taste, texture and overall acceptability are presented in Table 4.7. Significant increase in flavor and texture was observed due to incorporation of mango kernel flour. The summary of results and comparison of Control, MKF<sub>1</sub>, MKF<sub>2</sub> and MKF<sub>3</sub> were presented in figure 4.7

**Table 4.7: Sensory qualities of biscuits incorporated with MKF**

Types of biscuit	Hedonic Scales				
	Color	Flavor	Taste	Texture	Overall acceptability
Control	7.43±0.98 <sup>a</sup>	6.71±0.75 <sup>c</sup>	7.14±0.69 <sup>b</sup>	5.57±0.78 <sup>d</sup>	6.48±0.79 <sup>c</sup>
MKF <sub>1</sub>	7.43±0.98 <sup>a</sup>	7.29±0.48 <sup>b</sup>	7.28±0.48 <sup>b</sup>	6.85±0.69 <sup>c</sup>	7.0±0.58 <sup>b</sup>
MKF <sub>2</sub>	7.85±0.09 <sup>a</sup>	8.42 ±0.53 <sup>a</sup>	8.15±0.69 <sup>a</sup>	8.14±0.69 <sup>a</sup>	8.43±0.53 <sup>a</sup>
MKF <sub>3</sub>	5.85±0.69 <sup>c</sup>	6.14±0.89 <sup>c</sup>	6.14±1.07 <sup>c</sup>	8.29±0.48 <sup>a</sup>	6.43±0.97 <sup>c</sup>

All values are means ± SD. a-d Means not sharing a common superscript letter with in a column are significantly different (P > 0.05)

Whereas: 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4 =dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely



**Figure 4.7: Comparison of sensory qualities of biscuits.**

## **Chapter- 5: Discussion**

The seed kernel obtained after removal of the hard seed coat, is rich in carbohydrates, fat, minerals and protein. The kernel could potentially serve as a replacement for cereal flour in diets of human beings, animals and poultry. Manufacture of useful products and by-products from this waste would not only result in reducing the cost of production of main products, but also solve the problem of waste disposal.

The results of experiments conducted are discussed in this chapter:

### **5.1 Evaluation of physicochemical and nutritional properties of MKF and blend flour**

Study of the proximate composition of the flour is a prerequisite for any value addition programmed. Hence under this experiment the physical, chemical, nutritional and functional properties of mango seed kernel were evaluated.

#### **5.1.1 Proximate composition of MKF, wheat flour and blend flour**

The proximate constituents of the kernel flour evaluated were moisture, ash, protein, fat, fiber, and carbohydrate and the variation between varieties in proximate composition was significant. Proximate composition varied from variety to variety, but the mango kernel grown in Chittagong were similar to values obtained by Sunday (1999b); and Abdalla et al., (2007) with respect to moisture, crude fiber, and total ash.

Higher values were reported by Fowomola (2010) for crude protein (10.06%), crude oil (14.80%), ash (2.62%), crude fiber (2.40%), carbohydrate (70.12%) and energy (453 kcal) than the value obtained for sample MKF of crude protein (6.25%), crude fat (8.77%), ash (1.79%), crude fiber (3.26), carbohydrate (69.9%) and energy (396.96 kcal). The moisture content of the flour observed in the study corroborates with the findings of Abdalla et al., (2007). However, the crude protein (6.25%) was higher than the value obtained by Sunday (1999b) and lower than Abdalla et al., (2007) which were 5.3 g/100g and 6.7 g/100g respectively. Seleim et al., (1999) had reported that though the content of protein in the kernel flour was not very high the quality of protein was good because it was rich in all essential amino acids.

Dhingra and Kapoor (1985) had observed that mango kernel yield 6-12% of solid edible fat which is in general agreement with the results (crude fat 8.77%) obtained in the present study. Bose et al., (2001) and Zein et al., (2005) observed about 7.5-8.8% and 11% fat content in mango flour respectively. Dhingra and Kapoor (1985) further opined that the kernel fat could be used as a substitute of cocoa butter for making chocolate. Studies conducted by Rukmini and Vijayaraghavan (1984) had indicated that mango seed kernel fat is promising and a safe source of edible oil, is nutritious and nontoxic so that it could be substituted for any solid fat without any adverse effect.

Table 4.1 also indicated that the energy value of raw mango kernel were higher (396.96kcal) as compared to mango kernel flour and wheat flour.

### **5.1.2 Physical and Functional properties of flour samples**

Evaluation of the physical and functional properties of the flour is important since it indicates the suitability of the flour in manufacturing of dough and baked products. The functional properties studied (table 4.2) include oil absorption capacity, bulk density, water absorption and pH.

The bulk density value of mango kernel flour grown in Chittgong was higher (0.767 g/ml) than that obtained by Sunday (1999a) for MKF (0.50 g/ml) of Ikanekpo varieties grown in Nigeria.

The analysis showed that blending of MKF with WF significantly reduces the oil absorption capacity. Because oil absorption capacity was higher for wheat flour (control), which is 1.10 ml/g, and the lower was for mango kernel flour (MKF), which is 0.87 ml/g and both values were slightly different that founded by Legesse and Emire (2012).

The differences in the values of water absorption between wf, mkf and blend flour were not significant ( $P>0.05$ ), it ranged from 3.45 to 3.92. A high water absorption index is indicating suitability of flour for dough preparation and dough quality.

The pH value of the kernel flour was 5.5, which were proximate with the results of pH value obtained by Sunday (1999a), where pH=5.0.

## **5.2 Utilization of mango seed kernel flour for preparation of Biscuit**

The results of the experiment have revealed that MKF is rich in starch, fat and minerals. When MKF is blended with cereal flours the starch, fat and mineral fraction would improve the nutritional quality of the flours. The flour blends could be used as protein supplements and functional ingredients in human diet. Hence value addition studies were taken up with composite flour containing MSKF and cereal (rice, wheat and maize) flours. Production of baked goods by partial substitution of wheat with non wheat flours have been previously reported in literature.

Biscuits were prepared by incorporation of MKF and refined wheat flour in different proportions -10:90, 20:80 and 30:70 respectively.

### **5.2.1 Proximate composition of biscuits:**

Proximate composition of biscuits provide in Table-4.3. As indicated in Table 4.3 the analysis showed that there were no significant ( $P > 0.05$ ) differences on the moisture, carbohydrates and energy value of the biscuits containing mango kernel flour.

The protein and ash content of biscuits was significantly ( $P < 0.05$ ) decrease as the incorporation level of mango kernel increases. The crude protein values of biscuit samples are in the range of 6.16-5.55% which were lower than Ashoush and Gadallah (2012).

The fat and fiber contents of biscuits were increased as blending ratio of mango kernel flour increases. The fat content of the biscuits (7.25-8.16%) containing MKF observed in the study corroborates with the findings of Ashoush and Gadallah (2012).

### **5.2.2 Physical measurements of biscuits:**

The effect of replacing 10, 20 and 30% of wheat flour with MKF on physical properties of biscuits was studied and the data are presented in table - 4.4. The results showed that all selected MKF treatments caused significant increase in biscuit diameter as compared with 60.41 mm for control. The highest diameter (68.01 mm) was found by MKF<sub>3</sub> at without significant difference with 20% MKF. The higher thickness (7.90 mm) was recorded by MKF at 20%.



There are several views on the mechanisms by which the spread ratio of cookies is reduced when wheat flour is supplemented with non-wheat flours (Giami et al., 2005; Sudha et al., 2006). No significant difference was found in spread ratio between biscuit containing 20 and 30% MKP. This observation is agreement with those obtained by Ajila et al., (2007).

### **5.2.3 Mineral contents of biscuits:**

Results of the present study revealed that MKF has appreciably high levels of calcium, iron and potassium which are important macro minerals required for vital functions of the body. Mineral contents depend on the varieties. Calcium content ranged from 53-69 mg/100g. Phosphorus content is higher in mango kernel flour (Elegbede et al., 1995). The value of phosphorus and iron in biscuits were revealed in table-4.6. Iron is an important micronutrient which can be fortified in biscuits from MKF. Calcium (69.17 mg) and Iron (6.9 mg) content was found to be higher in MKF<sub>3</sub>. Phosphorus content was higher in MKF<sub>1</sub> (45.3 mg).

### **5.2.4 Microbiological investigation:**

Microbiological analysis of the prepared product was conducted to study the acceptance of the prepared products. *Escherichia coli* and *Salmonella sp* were absent in samples (table- 4.5).

### **5.2.5 Sensory characteristics of biscuits:**

Results of sensory attributes in terms of color, flavor, taste, texture and overall acceptability were presented in table-10. Biscuit prepared with 20% MKF showed maximum color mean score of 7.85, which was the highest obtained among the type of biscuit. Lower color mean observed in MKF<sub>3</sub>. This is due to the fact that as blending ratio with mango kernel flour increases, this in turn affects and led to the color change of the biscuits to darkness.

Flavor, texture and Overall acceptability were also highest for 20% mango kernel flour blend biscuit. The highest value of texture found in the 30% mango kernel flour blend biscuit.

The sensory results reported in this study are similar compared to the research finding of Sunday (1999b) who reported lowest sensory scores of flavor as incorporation levels of mango kernel flour increases.

## Chapter-6: Conclusions

The mango kernel flour is rich in starch, fat, minerals and also contains good quality protein and hence nutritive value of the food products would be very much enhanced by incorporation of processed kernel flour in food products. The processed mango seed kernel flour would be an ideal and cost effective substitute for enriching cereal flours in food products.

Physiochemical, nutritional and functional properties of flour extracted from mango seeds were studied. From the present study it can be concluded that blends containing up to 30% mango kernel flour were suitable for the development of biscuits. Beyond 30% blends of mango seed kernel flour, the color of the biscuits become darken attributable to the Maillard browning reaction, presence of high fiber composition and baking process parameters.

Mango seed kernel flour could also be used as a potential source for various bakery products and functional food ingredients. Furthermore, it can be processed into vital foods including nutraceuticals and therapeutic functional food products. It can also be considered as a functional food since it contains acceptable level of tannin even after processing. Most human diseases like cardiovascular disease, cancer, cataracts, age related muscular degeneration, rheumatoid arthritis and a variety of degenerative diseases are associated with free radical production and metabolism. Tannins can modulate this mechanism and improve the resistance against various diseases.

Utilization of mango seed kernels for commercial purposes can reduce the environmental pollution which resulted as by-product from mango processing industries; and contribute to food security by converting waste to valuable food products especially to Bangladesh context where mango fruit is abundant.

## **Chapter-7: Recommendations and Future perspectives**

Biscuits are the most popularly consumed bakery items in the world. Some of the reasons for such wide popularity are their ready to eat nature, affordable cost, good nutritional quality, availability in different tastes and longer shelf life.

Utilization of MKF in biscuit production increases its nutritional quality and also decreases the production cost. This process of utilization of mango seed will reduce the waste production. Now it is important to spread the technique.

More research activities have to be done in optimization of baking condition. The research work need to be carried out on different process for reducing tannin content in MKF and some important physical properties such as falling number, color grade etc.. Advanced research studies of mango seed kernels for utilization as industrial product, active ingredients characterizations, phytochemicals profile, antimicrobial activity, antioxidant activity via application of various processing for food and medicinal purposes are recommended.

However, the research work need to be carried out for storage stability of the MKF flour and product prepared from blend flour. Vitamin and minerals that are not analyzed in this study would be analyzed.

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**Appendix A: Photo Gallery**



**Fig: Mango Kernels**



**Fig: Blanching of kernels**



**Fig: Mango kernel flour**



**Fig: Biscuits of Blend flour**



**Fig: Biscuits of Blend flour**



**Fig: Baking of biscuits**



**Fig: Mineral Analysis by AAS**



### Appendix B: Sensory evaluation of biscuits

Name of Tester.....

Date.....

Please judge these samples and check how much you like or dislike each biscuit samples and overall acceptability. Use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample.

Hedonic Rating	Appearance				Colour				Flavour				Consistency				Overall Acceptability			
	Sample				Sample				Sample				Sample				Sample			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Like extremely																				
Like moderately																				
Like Slightly																				
Neither like nor dislike																				
Dislike slightly																				
Dislike moderately																				
Dislike very much																				
Dislike extremely																				

N.B. Overall scale used: 9= like extremely; 8= like moderately; 6= like slightly; 5= neither like nor dislike; 4= dislike slightly; 3= dislike moderately; 2= dislike very much; 1= dislike extremely

Signature of Judges



### **Brief Biography**

Taslima Noor passed the Secondary School Certificate Examination in 2007 and then Higher Secondary Certificate Examination in 2009. She also received Bachelor of Food Science and Technology (BFST) degree from Faculty of Food Science and Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong. Now, she is a candidate for the **MS degree in Food Chemistry and Quality Assurance** under the Department of Applied Chemistry and Chemical Technology of same faculty.

Her research interests are in the areas of Product development, Natural preservatives, Bio preservation of food, Food Safety and Food toxicology.