PHYSICAL PROPERTIES, PROXIMATE COMPOSITION AND ANTI-OXIDANT PROPERTIES OF WATER CHESTNUT (*Trapa bispinosa* Roxb.) FLOUR AND QUALITY EVALUATION OF COOKIES

A THESIS

BY

DIPA RANI ROY

Registration No. 1805162

Session: 2018-2019

Thesis Semester: January-June, 2019

MASTER OF SCIENCE (M.S.)

IN

FOOD SCIENCE AND NUTRITION

DEPARTMENT OF FOOD SCIENCE AND NUTRITION
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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DEPARTMENT OF FOOD SCIENCE AND NUTRITION
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The author

ABSTRACT

The aim of this study was to evaluate the physicochemical properties, antioxidant activities of water chestnut flour (WCF) and the cookies made from it. Further the present study also investigated the sensory evaluation of cookies prepared from water chestnut flour blending with different proportion (20%, 40%, 60%, 80% and 100%) of wheat flour. The chemical analysis performed by using AOAC and other standard methods. Methanol extract of water chestnut flour was used to analyze DPPH (2.2-diphenyl-1-picrylhydrazyl), and FRP (Ferric reducing power). Gluten analysis of water chestnut flour showed that the sample did not contain any gluten. The present study exhibited the water chestnut flour contained 9.77% Moisture, 4.33% Protein, 0.33% Fat, 81.59% Carbohydrate, 1.51% Crude Fiber, and 2.47% Ash. On the other hand, value of total phenolic content and flavonoid content were found to be (4.27 mg of GAE/g dry weight), and (0.21 mg of QE/g dry weight) respectively in water chestnut flour. The antioxidant activity was found to be (52.38% inhibition of DPPH) and FRP (137.51 mg $FeSO_4.H_2O$/g) of WCF. Sensory analysis indicated cookies made from 100% water chestnut flour had fair acceptability which could be due to their characteristic flavor however cookies made from 20 % WCF was best accepted by the panelists. Thus, water chestnut flour serves both as a gluten free as well as antioxidant rich flour for production of cookies.

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LIST OF ABBRIVIATIONS

BD(L)	= Bulk Density (Loose)
BD(P)	= Bulk Density (Packed)
DPPH	= deoxyribonucleic acid
Fe	= Iron
FRAP	= Ferric Reducing Antioxidant Power
g	= Gram
GAE	= Gallic Acid Equivalent
mg	= Milligram
ml	= Milliliter
OAC	= Oil Absorption Capacity
ppm	= Parts per million
rpm	= Revolution per minutes
SD	= Standard Deviation
S	= Solubility
SP	= Swelling Power
TAC	= Total Anti-oxidant Capacity
TFC	= Total Flavonoids Content
TPC	= Total Phenolic Content
TRAP	= Total Reactive Anti-oxidant Potential
WAC	= Water absorption capacity
WCF	= Water Chestnut Flour
WF	= Wheat Flour
µg	= microgram

CHAPTER 1

INTRODUCTION

One of the major challenges in baking industry is the production of gluten free products. Gluten, important for dough development, is usually associated with gluten intolerance and gluten sensitivity that have recently been categorized as two different medical conditions and are associated with the consumption of gluten protein. Usually those gluten free sources are preferred for incorporation in bakery products that are rich in starch. Keeping in view, various under-utilized food sources have been incorporated in bakery products. Incorporation of buckwheat, amaranth, quinoa and barley has been thoroughly studied due to absence of gluten and rich antioxidant profiling. Among such gluten free sources is water chestnut that is not only rich in starch but has also been reported for its high flavonoid content (Shafi M. *et al*., 2016).

Water chestnut (*Trapa bispinosa* Roxb) is an edible aquatic angiosperm locally known as "Singhara". It is one of the important annual aquatic warm season crops. It is a floating plant, found commonly on the water surface of lakes, tanks and pools throughout Indian Sub-continental specially in India, Pakistan and Bangladesh (Puste,2004; Takano and Kadono,2005).

Recently the cultivation of water chestnut is becoming more popular in Bangladesh due to its easy grow, low cost production, good profit and to meet up the demand for more food production. Several varieties are grown in Bangladesh among which *Trapa bispinosa* Roxb. and *Trapa natans* L. are important. *Trapa bispinosa* has two varieties ,one is red (Leaf, Petiole and Fruit) and the other is green (Leaf, Petiole and Fruit),each of the fruit is large in size having four dull spines.

Water chestnut is an important commodity in food industry because of its unique taste (Parker and Waldron,1995).Water chestnuts can be used in a variety of recipes because they have a starchy taste that is fairly neutral. It also has a firm and crispy texture which adds to their appeal as an ingredient in stir-fries, salads or any meals where the vegetables to be used must have a crunchy consistency. The fruit is eaten raw at tender stage and sometimes after boiling and roasting. It is mainly consumed in the form of cooked vegetables, flour or in the shape of sweet dishes of many kinds. It compares well with other foods and is a good source of carbohydrates, proteins and essential minerals.

The dark-brown corns (whole fruit) are peeled before cooking or canning. The bulk of the edible region consists of starch-rich, thin walled storage parenchyma similar in the appearance to potato, interspersed with vascular strands. However, in the contrast to potato, water chestnut is notable for its ability to maintain a firm and crunchy texture after considerable heat treatment during canning and cooking. This property is attributed to the lack of cell separation during cooking (Loh *et al*.,1982; klockeman *et al*.,1991). Usually, the fruits are washed, peeled, sliced and packaged before commercially sold. However, minimally processed fresh products have relatively short shelf life, because of large amount of tissue disruption and increased metabolism that lead to rapid onset of enzymatic browning.

According to Kusum and Chandra (1980) the nutritive value of the fruit is not less than that of wheat. It also contains plentiful B vitamins (including B1, B2, B5 and B6), E, A and ascorbic acid. Water chestnut (*Trapa bispinosa* Roxb) possess strong antioxidant, antimicrobial and anticancer activities, which have been attributed to their bioactive components, such as polyphenols, flavonoids and alkaloids (Yu *et al*. 2013; Chiang and Ciou 2010). Water chestnut extracts are also reported to have high inhibitory activity against glycolytic enzymes such as α-amylase that inhibits blood glucose elevation and a unique feature of reducing insulin secretion and hence can be effective food additives in managing type 2-diabetes (Yasuda *et al*. 2014). Recent researchers are focusing on alternatives that can cut down production cost as well as improve nutritional as well as nutraceutical status of food items (Baba *et al*. 2016).

Water chestnut is available in abundance in south East Asia, China and northern India during the months of November to March and can be a good option to achieve such objectives. In addition, use of natural sources of antioxidants as food additives is gaining interests due to economical and safety concerns associated with synthetic additives. Water chestnut fruit being a rich source of starch with no gluten, its flour can be used to replace wheat flour for the production of gluten free products. It is also used to make colored powder and dye (Kusum and Chandra,1980).

In addition, flour production being a size reduction process may alter the structure, surface area and functional properties of flour resulting in some new applications (Chiang and Ciou 2010). Although some basic work regarding characterization of water chestnut flour and production of water chestnut cookies (Sarabhai and Prabhasankar

2015; Singh *et al*. 2011) has been carried out but the antioxidant potential of water chestnut flour and effect of baking on its antioxidant activity during cookie production has not been reported so far.

Some research is going on in several institutes of our country with the emphasis on the callus induction and in vitro organogenesis and thus plant regeneration, higher production, development of new varieties, control of the diseases and there by higher production of fruits. However data available on the physicochemical parameters, enzyme and antimicrobial activities of water chestnut produced in Bangladesh is quite scanty. Only a limited work was done on the physicochemical properties on water chestnut in Bangladesh (Majid,1986; Irfanullah, 2002).

Recently Alfasane *et al* (2008-2009) have studied the biochemical composition of the seeds of *Euryale ferax* Salisb and *Nelumbo nucifera* Gaertn. The natural habitat of the plant has also been decreasing at a high rate.

Water chestnut may contribute a lot in the nutrition of our Bangladeshi people by providing essential nutrients. Therefore keeping all these in mind in this study, an attempt was made to investigate the physio-chemical properties of locally available varieties and develop gluten free product using water chestnut flour.

Thus the specific objectives of the present study were to

i. analysis the physical properties of water chestnut flour such as bulk density, water and oil absorption capacity, swelling power and solubility,
ii. analysis of proximate composition and anti-oxidant properties of water chestnut flour and cookies made from it,
iii. to measure the acceptability of the prepared products by conducting their sensory analysis.

CHAPTER 2

LITERATURE REVIEW

Nature itself is a complete store-house of remedies to cure and prevent almost all ailments of humans. As the population is increasing rapidly, inadequate supply of drugs, high cost of treatment, side effects along with drug resistance has been encountered in synthetic drugs which has lead to an elevated emphasis for the use of plants to treat human diseases. The healing powers of traditional herbs have been realized since antiquities (Bhatiwal S. *et al*, 2012)

In wake of growing demand of the consumers for natural foods having good therapeutic values, water chestnut offers excellent opportunity. The consumption values of the fresh fruit are probably linked to the high nutritional and organoleptic value and also to the increasing interest of consumers towards organic products (Singh G.D.,Singh S.*et.al.*, 2010).Water chestnut (*Trapa bispinosa* Roxb) is a floating annual aquatic plant that is commonly found in the shallow stagnant waters of tropical and sub-tropical countries. Like most other macrophytes, these are self-growing plants that grow in slow moving water up to 5m deep and are native to warm temperate parts of Asia and Africa. The fruit (nut) of water chestnut is eaten by humans in raw or cooked form (Khan MS, Halim,1987). When the fruit has been dried, it is ground to a flour called singhara atta which is used in many religious rituals and can be consumed as a phalahar diet on the Hindu fasting days as substitute for cereal, in Indian traditional festive 'Navratri'.(Chandana M, Mazumder R *et al*.,2012).

Trapa bispinosa is an annual aquatic plant found in tropical, sub-tropical and temperate zones of the world. Their natural range of growth includes Southern Europe, Africa and Asia. It has been grown in Europe since Neolithic times. It is commonly used as food by ancient Europeans as an easy growing plant; it has become neutralized in part of USA since it was first introduced into North America in 1874. It was found in slow moving rivers, ponds, lakes and damps and is widely cultivated in Asia. It favors nutrient rich water with pH range between 6.7 and 8.2 and alkalinity between 12 and 128mg/l of calcium carbonate (Bhatiwals S, Jain A *et al*.,2012).

Figure 2.1 Water Chestnut

In Bangladesh, there exists a variety of water chestnut locally known as paniphal or singhara-an edible aquatic angiosperm. It belongs to Trapaceae family in the genus Trapa and species *Trapa bispinosa* Roxb. The interesting features of paniphal are the color and shape of its outer cover in which the nut encased. The nut is covered with a thick jet-black outer cover shaped like a horn. The outer cover is hard,making it quite difficult to peel off to obtain the white nut inside (Alfasane M.A., Khondker M. *et al*.,2011).

2.1 Botanical description (Ghani A., Haq S.S. *et al*.,2010)

Common names

English: water chestnut, water caltrop

Bangla: singhara, paniphal.

Synonyms

Trapa bispinosa Roxb.

Trapa natans

Trapa natans var. *natans* L.

Trapa natans var. *bispinosa* Roxb.

Biogeography and ecology

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Class: Magnoliopsida

Order: Myrtales

Family: Trapaceae

Genus: Trapa

Species: *Trapa bispinosa* Roxb.

Botanical description

It is an annual aquatic floating herb found in lakes and ponds. Floating leaves are rhomboid in shape, 2–6.5cm in diameter, dark green above and reddish purple beneath, broader than long, denticulate, dentate, serrate or incised with entire base, apex acute red, and densely pubescent or villous beneath. The reddish green leaves are villous on the dorsal side, are about 5 to 8cm long, and have hairy petioles from 10 to 15cm in length. The submerged leaves are laterally dissected into capillary segments.

Flower

Flowers are axillary, white in color, and with a solitary peduncle. They open above the surface of the water towards the afternoon. After pollination, the flowers submerge to facilitate fruit formation.

Fig. 2.2 Flower and Leaf of Water Chestnut.

Fruit

It is obovoid, triangular with two horns and is about 2cm in diameter. One seeded nut, has very unequal cotyledons and a top-shaped drupe. The fleshy pericarp covers a large

2–4 horned, stony endocarp. (Karmakar *et al*., 2011). It is green in fresh condition, but after drying it becomes blackish; pulp of the fruit is whitish, sweet in taste.

Fig. 2.3 Fruits of Water Chestnut

Stem

The stem anchors into the mud by numerous branched roots and extends upward to the surface of the water. Cord like stems are spongy and buoyant and can reach lengths of up to approximately five meters (16 feet) (Adkar P.,Dongare A *et al*,2014).

2.2. Historical Perspectives

Trapa bispinosa had been introduced from Europe as an ornamental plant. Dispersal is limited because of the large, sinking nuts, but water chestnut has persisted and spread in the Northeastern states. In the Chinese Zhou Dynasty, water caltrop was an important food for worship as prayer offerings. The rites of Zhou (2nd century BC) mentioned that a worshipper should use a bamboo basket containing dried water caltrops. In India it is known as singhara or paniphal (eastern India) and is widely cultivated in fresh water lakes.

The *Trapa bispinosa* is native to Eurasia. It was first introduced to North America in the 1870s, where it is known to have been grown in a botanical garden at Harvard University in 1877. The plant had escaped cultivation and was found growing in the Charles River by 1879. Water chestnut can now be found in Connecticut, Maryland, Massachusetts, New York, Pennsylvania, Vermont and Virginia and in the Canadian Province of Quebec in a tributary of the Richelieu River (Ling Cao 2009). Water chestnut has occurred repeatedly in tributaries of the Chesapeake Bay, where plants were first discovered in the 1920s. Pennsylvania has reported populations in the Lower Susquehanna, areas around Philadelphia, and in isolated lakes and ponds in Montgomery and Bucks Counties

(Figure 4). Most recently, a population was reported in the Upper Delaware River (MD SeaGrant 2007, Perkiomen Watershed Conservancy, personal communication).

2.2.1 Habitat

Trapa bispinosa Roxb. (Family: Trapaceae) is native to India. The fruit is commonly known as "Paniphal." It grows abundantly in the lakes of Kashmir, India. The plant is commercially cultivated in tropical parts of the world such as Pakistan, Sri Lanka, Ceylon, Indonesia, and Africa. The plant is also abundant in Indonesia, southeast Asia, and the Southern part of China and in the eutrophic waters of Japan, Italy, and tropical America. It has become naturalized in a few places in the Eastern United States (U.K.,Karmakar *et al*,2011). It is commercially cultivated across different parts of India for its consumable seasonal fruit commonly known as singhara which is a good source of nutrition having considerable amount of carbohydrate, protein, and vitamins. *Trapa bispinosa* Roxb plant floats just beneath the water surface and thus forms a thick mat in the water column. Only its upper leaves float over water surface in an artistic radial pattern with swollen, air-filled petioles that keep the upper part of the plant afloat (U.K.,Karmakar *et al*, 2011).*Trapa bispinosa* Roxb was first observed in North America, growing "luxuriantly" in Sanders Lake, Schenectady, New York, in 1884. The plant subsequently spread to many other areas in the Northeastern United States including Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, Pennsylvania, Vermont, Virginia, and Washington D.C. The plant is now present in the Great Lakes Basin and recently has been found in Quebec, Canada.

2.3 Cultivation and collection

Trapa bispinosa seedlings are transplanted in May/June in a perennial pond. These plants make use of the available organic matter for their growth. Seeds germinate in the spring with each seed producing 10 to 15 rosettes and each rosette capable of producing up to 20 seeds. The plant starts to produce hard , nut-like seeds in July with the seeds ripening in about a month. Over winter of populations is accomplished when the mature, greenish brown seeds sink to the bottom where they can remain viable in the sediment for up to 12 years. The plant spreads either by rosettes detaching from their stems and floating to another area or more often by the nuts being swept by currents or waves to other parts of the lake or river.(O'Neill, C.R. et al.,2006). *Trapa bispinosa* fruits ripen in winter and are harvested from November to January.(Singh G.D., Singh S.*et al*.,2010)

2.4 Notable characteristics

Matures seeds are very hard with sharp points easily capable of piercing light footwear.

2.4.1 Historic Vectors

Escape from horticultural collections, sold as ornamental plant, escape from the aquarium trade, hitchhiker in ballast water.

2.4.2 Current Pathways/Vectors

Seed dispersal by animals, human dispersal via the ornamental plant trade and escapes from ornamental ponds (Perkiomen Watershed Conservancy, personal communication), hitchhiker on recreational equipment (Eyres 2009).

2.4.3 Preferred Habitat

Trapa bispinosa Roxb grows best in shallow (less than five meters deep), nutrient-rich lakes and slow-moving streams and rivers with soft muddy bottoms. It is generally found in waters with a pH range of 6.7 to 8.2 and alkalinity of 12 to 128 mg/L of calcium carbonate (Ling Cao 2009).

2.4.4. Threats

Water chestnut out-competes native plants for sunlight and is a fierce competitor in shallow waters with soft, muddy bottoms. This aggressive species is a prolific reproducer. One acre of water chestnut can produce enough seeds to cover 100 acres the following year (MD Sea Grant). Uncontrolled, it creates nearly impenetrable mats across wide areas of water, thus limiting the passage of light into the water, a critical element of a well-functioning aquatic ecosystem. At die off, it reduces oxygen levels, which may increase the potential for fish kills. *T. natans* is of little value to wildlife (O'Neill 2006).

Water chestnut infestations create havoc for boating and recreational areas. The dense mats make navigation difficult, while the spiked seeds, capable of puncturing shoe leather, are a danger to bathers and beach users. When the plant occupies a site, most recreational activities such as swimming, fishing from the shoreline, and the use of small boats are eliminated or severely impeded. (Ling Cao 2009, MD DNR 2010).

2.5 Phytochemistry

Trapa bispinosa (singhara) contains many organic and inorganic constituents which are mentioned below.

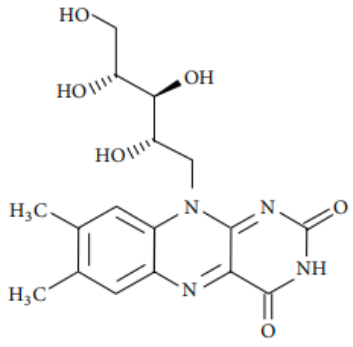
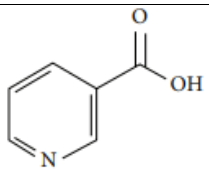
2.5.1 Inorganic Constituents

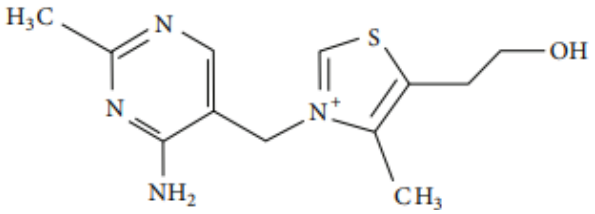
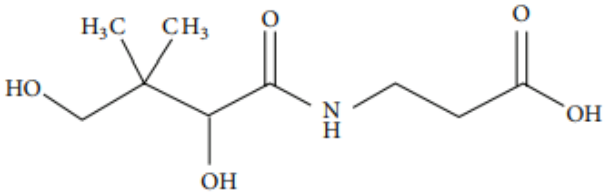
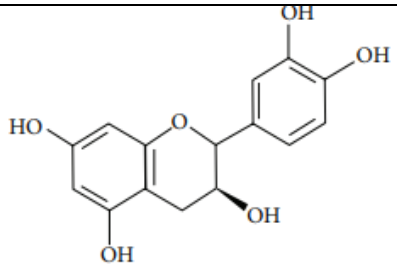
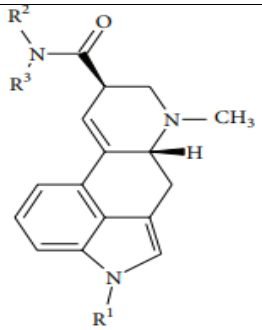
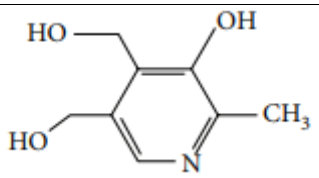
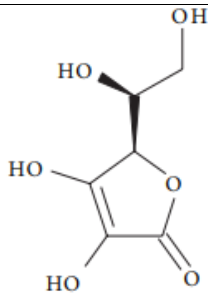
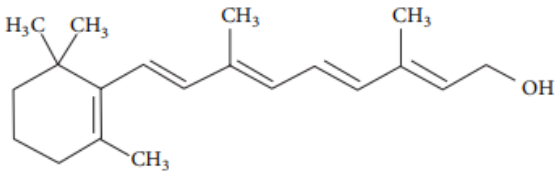
Acids, minerals, calcium, phosphorus, iron, copper, manganese, magnesium, sodium and potassium. (Jolly R.S.,2017)

Minerals	Amount
Calcium	17.6 grams
Zinc	0.4 grams
Iron	0.7 grams
Sodium	0.8 grams
Potassium	468 miligrams

2.5.2 Organic Constituents

It contains carbohydrates and vitamins, namely, Vitamin B-complex (thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid), vitamin-C, vitamin-A, D-amylase, amylase, and considerable amount of phosphorylase (Khare C.P., 2007). Cycloeucalenol, ursolic acid, and 2β,3α,23-trihydroxyurs-12-en-28-oicacid (Song M.C., Lee D.Y. *et al.*,2007).

Name of constituents	Chemical structure
Riboflavin (vitamin B2)	
Nicotinic acid/Niacin (vitamin B3)	

Thiamine (vitamin B1)	
Pantothenic acid (vitamin B5)	
Flavonoids	
Alkaloids	
Pyridoxine (vitamin B6)	
Ascorbic acid (vitamin C)	
Retinol (vitamin A)	

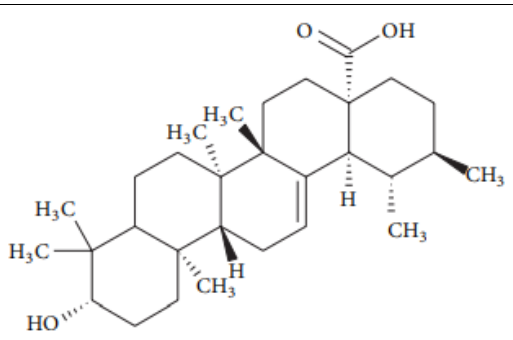
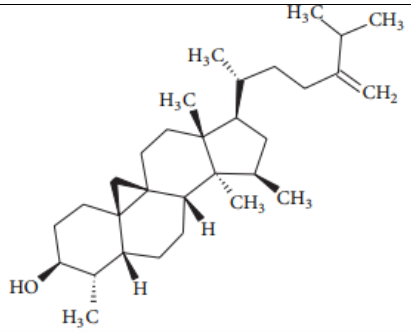
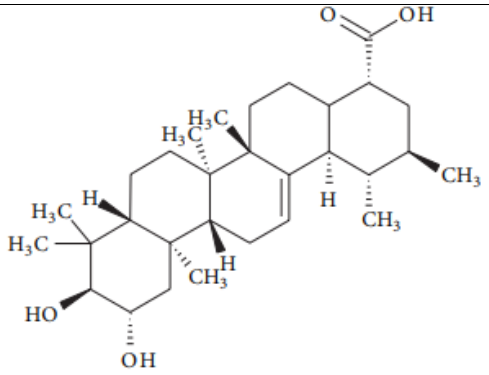
Triterpenoids (ursolic acid)	
Triterpenoids (cycloeucalenol)	
2β, 3α)-2,3-Dihydroxy-urs12-en-28-oic acid	

Fig 2.4: Chemical constituents present in the plant of *Trapa bispinosa*.

2.6 Nutrition benefits (Jolly R.S.,2017)

- Gluten free
- Low in fat
- Cholesterol-free
- Low in sodium
- High in potassium
- Rich in minerals including calcium, iron, zinc and phosphorus
- Contains moderate amount of fiber
- Good source of energy.

2.7 Pharmacognostic characters

Trapa bispinosa contains a great quantity of non-nutritional antioxidants, such as flavonoids, flavones, and total phenol contents. Flavonoids are present in plant tissues such as fruits, vegetables, nuts, seeds, and leaves, in relatively high concentrations. Flavonoids act as natural antioxidants. Phytochemical screening of seed extract of *Trapa bispinosa* fruits reveals the presence of carbohydrates, saponins, phytosterols, fixedoils and fat ,while the pericarp extract of the fruits of *Trapa bispinosa* revealed the presence of tannins, flavonoids and glycosides alkaloids, saponins, steroids, and phenolic compound.(Patel S.,Banji D.*et al*.,2010).The kernel is delicious and contains carbohydrates, proteins and essential minerals. It also contains plentiful B vitamins (including B1, B2, B5, and B6), E, A, and C vitamins. Seeds also contain thiamin (Singh G.D., Singh S. *et al.*,2010).

2.8 Pharmacological activity

Analgestic activity

The analgetic activity of methanolic extract of root of *Trapa natans* L.var. *bispinosa* Roxb. by evaluating 200mg/kg and 400mg/kg dose on mice against the standard drug pentazocine at a dose of 30mg/kg. Both doses are found to produce significant ($p<0.01$) analgesic activity (Agrahari A.K., Khaliquzzama M.,2010).

Anti-inflammatory activity

Both pericarp and seed extract of fruits of *Trapa bispinosa* for anti-inflammatory activity by carrageenan induced hind paaw edema with 200mg/kg and 300mg/kg dose. Pericarp shows more potent action than seed (Patel S.,Banji D. *et al*.,2010).

Anti-diabetic activity

The anti-diabetic activity of methanol extract of *Trapa bispinosa* fruit peels (METN) in STZ induced diabetes in Wistar rats. METN at the dose of 100 and 200mg/kg orally significantly ($p<0.001$) and dose dependently improved oral glucose tolerance, exhibited hypoglycaemic effect in normal rats and anti-diabetic activity STZ-induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group (Das P.K., Bhattacharya S. *et al.*,2011).

Anti-microbial activity

Anti-bacterial activity of different extracts of *Trapa bispinosa* fruit rind by agar disc diffusion method. Maximum anti-bacterial activity was observed against Gram negative bacteria. The best anti-microbial was with 1,4-dioxan extract and the least activity was with petroleum ether extract (Parekh J., Chanda S.,2007).

Morpho-physiological activity

Fasulo (2008) reported the ability of floating lamina of the rhyzophyta *Trapa bispinosa* to bioaccumulate Mn (>3000µg g-l) by means of phenolic compounds (Baldisserotto L., Ferroni L. *et al*.,2004).

Anti- ulcer activity

The anti-ulcer activity of 50% ethanolic extract of the fruits of *Trapa bispinosa* was studied on wistar rats using pyloric ligation and aspirin plus pyloric models by Kar (2010) (Kar D.M., Maharana L. *et al*, 2010).

Neuroprotective effect

Vyawahare (2010) reported that the hydroalcoholic extract (500mg/kg,po) of *Trapa bispinosa* decreased fluroscence product and increase in lipid peroxidation and restored glutathione peroxidase and catalase activity in cerebral cortex in the brain of female albino mice.(Ambikar D.B., Harle U.N. *et al*, 2010)

2.9 Ayurvedic properties (ayurvedicmedicinalplants.com,2011)

Rasa: Madhura, Kashaya. Rasa is a herbal medicine which helps to prevent facial, teeth, skin, hair and any other dis-order.

Guna: Guru, Snigdha.Guna helps to prevent gastric problem, ulcer and other inflammatory diseases.

Veerya: Seeta. It helps to recover male sexual and sperm disorders.

Karma: Vrushya.It helps to keep every human to look young and healthy naturally.

Vipaka: Madhura.It is the final taste of a drug which is encountered after exposure to digestive enzymes.It is comparable with the metabolism of the drug.

2.10 Usage

Food

The fruits are used either boiled or roasted or can be dried and ground into flour which is sometimes used as a substitute for arrowroot flour. The fruits are a good source of nutrition with 16% starch and 2% protein. When raw the fruits are juicy and crisp, again when cooked the flesh softens but it still remains crunchy. The kernels are good source of minerals, vitamins, carbohydrate, calcium, phosphorus, iron, copper, magnesium, sodium and potassium (Singh G.D., Singh S. *et al*, 2010).

Medicine

This is used in many Ayurvedic preparations as nutrient, appetizer, astringent, diuretic, aphrodisiac, cooling, anti-diarrhhoeal and tonic. It is also useful in lumbago, sore, throat, bilious affections, bronchitis, fatigues and inflammation. Plant pacifies vitiated pitta, burning sensation, hemorrhages, skin diseases, low back ache and general debility. Fruits are also used for making liniments for cure of rheumatism, sores and sun-burn. It is also said to have cancer-preventing properties. Stem is used in the form of juice in eye disorder (Singh G.D., Singh S. *et al*, 2010).

2.11 Adverse effect of Water chestnut

One should not consume water chestnut if she / he has constipation. Also should avoid consumption in excess as these seeds can cause bloating and abdominal pain (Jolly R.S.,2007).

2.12 Bakery product

Bakery products are the most popular food consumed by all age groups and are gaining popularity as processed foods because of their availability, ready to eat convenience, and comparatively good shelf life.

Celiac disease, an autoimmune disease caused by the interaction of gluten in genetically predisposed individuals (Marsh M.N., 1992), is common in areas of North India where wheat is a staple food (Mir, Gul *et al*. 2014). A strict gluten-free diet can fully restore health and improve the quality of life in patients with celiac disease and is therefore the basic line of treatment (Stern, 2008). There is a need for the development of a range of

gluten-free products as the demand for these products is increasing worldwide with the increase in the number of individuals diagnosed with celiac disease (Arendt, Dal Bello, 2008). Gluten-free products are developed by using flours that are gluten free, however, persons with celiac disease have a lower intake of fiber as compared to a control group of people on normal diet (Grehn, Fridell et al. 2001), it is imperative to keep in view the fiber content of the new gluten-free products.

Cookies prepared from water chestnut flour can also be used as a specialized product during Navratras and other sacred or fasting days observed frequently in India. Cookies have gained importance as a preferred way to use water chestnut flours as they are ready-to eat, provide a good source of energy, and are consumed widely throughout the world (Arshad, Anjum *et al*., 2007; Chavan, Kadam, 1993).

2.13 Gluten

Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin. Different wheat varieties vary in protein content and in the composition and distribution of gluten proteins. Collectively, the gliadin and glutenin proteins are referred to as prolamins which represent seed proteins insoluble in water but extractable in aqueous ethanol and are characterized by high levels of glutamine (38%) and proline residues (20%) (Wieser H., 2007).

Gluten is appreciated for its visco-elastic properties. It gives elasticity to dough (Penagini F.,Dilillo D. et al., 20130). Gluten is heat stable and has the capacity to act as a binding and extending agent and is commonly used as an additive in processed foods for improved texture, flavor, and moisture retention.(Kucek LK, Veenstra LD et al.,2015).

Gluten is prepared from flour by kneading the flour under water, agglomerating the gluten into an elastic network, a dough and then washing out the starch (Mooney P.,Sanders D.,2013).

2.13.1 Adverse reactions of gluten

Celiac disease

Celiac disease (CD) is a long term autoimmune disorder primarily affecting the small intestine caused by the ingestion of wheat, barley, rye, and derivatives, that appears in

genetically predisposed people of all ages. CD is not only a gastrointestinal disease because it may involve several organs and cause an extensive variety of non-gastrointestinal symptoms, and most importantly, it may often be completely asymptomatic.(World Gastroenterology Organisation Global Guidelines, 2017).

Added difficulties for diagnosis are the fact that serological markers (anti-tissue transglutaminase [TG2]) are not always present and many people may have minor mucosal lesions, without atrophy of the intestinal villi. (Bold J, Rostami K, 2011).CD affects approximately 1–2% of the general population, but most cases remain unrecognized, undiagnosed and untreated, and at risk for serious long-term health complications. People may suffer severe disease symptoms and be subjected to extensive investigations for many years, before a proper diagnosis is achieved.(Ludvigsson JF, Card T, Ciacci C, 2015).

Untreated CD may cause malabsorption, reduced quality of life, iron deficiency, osteoporosis, an increased risk of intestinal lymphomas, and greater mortality.CD is associated with some other autoimmune diseases, such as diabetes mellitus type 1,thyroiditis, gluten ataxia, psoriasis, vitiligo, autoimmune hepatitis, dermatitis herpetiformis, primary sclerosing cholangitis, and more (Ciclitira PJ, Swift GL, NasrI, Sanders DS, 2014).

CHAPTER 3

MATERIALS AND METHODS

The current study on "Physical properties, proximate composition and anti-oxidant properties of water chestnut (*Trapa bispinosa* Roxb.) flour and quality evaluation of cookies". The material and methods used for the study are described in this section.

3.1 Experimental site

This study was conducted in Food science and nutrition Laboratory, Food Processing and Preservation Laboratory and Food Engineering and Technology Laboratory under the Faculty of Engineering. Some physico-chemical analysis were also accomplished in the laboratories under the department of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University.

3.2 Materials

Water chestnut were purchased from the local market of Dinajpur and stored at 4°C till further use. Sugar, butter, eggs and synthetic food grade flavor (Foster vanilla) were also collected from Dinajpur local market. All other equipments and analytical grade chemicals were supplied from the laboratories.

After collection of water chestnut from local market, were washed, peeled them and cut into small pieces. The pieces were dried at 55°C for 20 hours on a perforated tray in a 2-stages fluidized bed dryer. Subsequently, after cooling they were ground separately in a blender to get flours. Flours were sieved and packed in double layer polyethylene films with sealing till further use.

3.3 Formulation of composite cookies

Cookies were prepared according to formula described by Jan et al. (2015). In this study, certain percentage WCF was incorporated to WF in cookies preparation. The final products were coded as 101 (100%WF and 0%WCF)), 123 (80%WF and 20% WCF), 231 (60%WF and 40% WCF), 321 (40%WF and 60% WCF), 456 (20%WF and 80%WCF), 564 (0%WF and 100%WCF).The ingredients used in the preparation of 100g dough are presented in Table 3.1

Table 3.1. Formulation of WCF supplemented biscuits (Total 100 g)

Ingredients	Amount of ingredients in different Samples					
	101	123	231	321	456	564
Wheat Flour(g)	40	32	24	16	8	0
Water Chestnut Flour(g)	0	8	16	24	32	40
Sugar(g)	20	20	20	20	20	20
Fat (dalda)(g)	20	20	20	20	20	20
Milk powder(g)	2	2	2	2	2	2
Baking powder(g)	1	1	1	1	1	1
Vanilla Essence(drops)	1-2	1-2	1-2	1-2	1-2	1-2
Salt(g)	0.5	0.5	0.5	0.5	0.5	0.5
Egg	16	16	16	16	16	16

3.3.1 Development of composite cookies

At first, the fat was mashed finely and pre-blended sugar was added to it. Egg, salt, milk powder and vanilla essence were added and mixed well. After that, the flours and baking powder were added and mixed well to produce dough. Then the dough was rolled into thin uniform sheet of 3 mm thickness. After sheeting, the sheet was cut out using a round biscuit cutter of 3 cm diameter. Then the cookies were baked at 180°C for 15 minutes by using baking oven. The prepared cookies were cooled at room temperature and packed for storage to use further.

Fig. 3.1 Cookies

3.4. Determination of pH (Faruk M.K.,Amin M.Z., *et al* 2012)

- **Extraction of juice from water chestnut (*Trapa* sp.):**
  About 70-90 g of water chestnuts (*Trapa* sp.) was taken in a mortar. The fruits were crushed thoroughly in a mortar with a pestle and then filtered through whatman filter paper. The filtrate was then centrifuged for 10 min at 3000 rpm and the clear supernatant was collected.
- **Preparation of standard buffer solution:**
  Buffer solution of pH 7.0 or 4.0 was dissolved in distilled water and made up to the mark of 100 ml with distilled water.
- **Procedure:**
  The electrode assembly of the pH meter was dipped into the standard buffer solution of pH 7.0 taken in a clear and dry beaker. The temperature correction knob was set to 28°C and the fine adjustment was made by asymmetry potentially knob to pH 7.0. After a wash the electrode assembly was then dipped into a solution of standard pH 4.0 and adjusted to the required pH by fine asymmetry potentially knob. The electrode assembly was rinsed, washed twice with distilled water, rinsed off with the juice of the water chestnut (*Trapa* sp.).The pH of the juice was noted.

3.5. Physical properties of water chestnut flour

3.5.1. Gluten presence test (Hand washing method)

The gluten presence test was done by Hand washing (AACC, 2010) method. For this 100gm flour and 86 gm water were mixed well for making dough. From this dough 50gm was taken for further experimental process. This 50gm dough was immersed into a beaker (500ml) with water and rested in water for 30 minute at room temperature.

Then the dough was taken in a thin clean cloth and hold the cloth carefully so that it could not bring outside at the time of washing. The dough was kneading gently in stream of tap water over cloth until starch and all soluble matter were removed. This process took 30 min.

To determine whether gluten was approximately starch-free, squeezed the dough falled into see thru glass containing perfectly clear water. When clear water appeared the kneading process was stopped.

The starch free dough was pressed as dry as possible between the hands, rolled into a ball, placed in a dish and weighed it as moist gluten. (Aquino Joanne,2014).

3.5.2 Bulk density (loose and pack)

Bulk density (loose and packed) was determined by the procedure as described by Mir et al. (2014). For loose bulk density, an empty and dried 50 ml measuring flask was weighed and flour sample was allowed to fall freely into it up to the mark, with gentle tapping. The flask was weighed again along with the sample. For packed bulk density, the same sample was tapped inside the measuring flask with the help of rubber pad, and more of the sample was added up to the mark before weighing. The results were reported as g/ml.

$$\text{Bulk density} = \text{Weight of water chestnut flour} / \text{Bulk volume.}$$

3.5.3 Water and oil absorption capacity (WAC and OAC)

Flour samples (1.0 g each) at ambient temperature were mixed with 10 ml distilled water or refined mustard oil and stored for 30 min. Samples were centrifuged (Eppendorf Centrifuge 5810R, Germany) for 10 min at 2000 g. The aqueous supernatant or clear oil obtained after centrifuging was decanted and the test tubes were inverted and allowed to drain for 5 min on a paper towel. By weighing the residue, WAC and OAC were calculated and expressed as percentage of water or oil absorbed per gram of sample, respectively (Beuchat et all,1975; Abbey and Ibeh,1988)

$$\text{Water absorption capacity} = \frac{\text{Wet sample wt} - \text{Dry sample wt}}{\text{Dry sample wt}} \times 100\%.$$

$$\text{Oil absorption capacity} = \frac{\text{Wet sample wt} - \text{Dry sample wt}}{\text{Dry sample wt}} \times 100\%.$$

3.5.4 Swelling power (SP) / Solubility (S)

For determining swelling power and solubility of the flour samples, known amount of dry flour (0.5 g) was dispersed in 50 ml of water and the dispersion was heated under mild agitation at 90 °C for 30 min. The gelatinized dispersion was then centrifuged

(3000g) for 15 min and the supernatant was decanted and dried at 100 °C up to a constant weight. The swelling power and solubility were determined using the given standard equations and the results were expressed as g/g of dry flour (Leach, H.W.,1959; Kinuma, Cuzuki.1967).

$$\text{Swelling power} = \frac{\text{Wt of sedimental paste (g)}}{\text{Wt of the sample (dry basis)(g)}}$$

$$\text{Solubility} = \frac{\text{Wt of soluable starch(g)}}{\text{Wt of the sample (dry basis)(g)}} \times 100\%$$

3.6 Antioxidant activity of WCF

3.6.1 Preparation of extract:

Each sample (0.3 g) was dissolved in 20 ml of 70% methanol. After stirring for 2 hours on a magnetic stirrer, it was centrifuged (3500 rpm) for 10 min. The supernatant was filtered and stored at −18 °C.

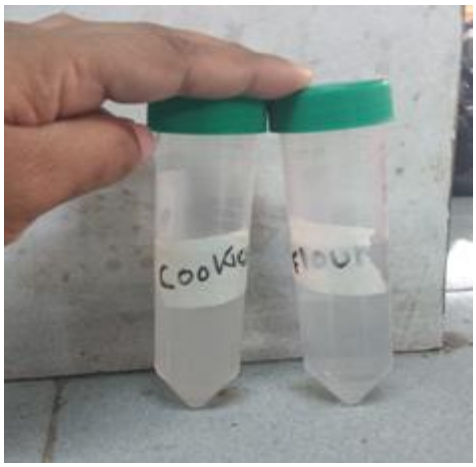


Fig. 3.2 Extract of flour and cookies of water chestnut

3.6.2 Determination of phenol content

Reagents preparation

i. **Gallic acid solution**

   Different gallic acid solutions were prepared having concentration ranging from 50µg/ml to 1000 µg/ml for plotting standard curve.

ii. **Folin-Ciocalteau reagent**

10 ml FCR was taken in a beaker and 900 ml of distilled water was added for 10 times dilutions.

iii. **7.5% Na_2CO_3 solution**

7.5 gm Na_2CO_3 was taken in a 100 ml volumetric flask and a small amount of distilled water was added in it and shaken to dissolve Na_2CO_3 and the volume was made up to the mark by adding water.

Total Phenol Content

Total phenolic content of water chestnut flour extract was measured using by spectrophotometric method using some modifications by applying the method involving Folin-Ciocalteu reagent as oxidizing agent and Gallic acid as standard.(Demiray *et al*,2009; Majihenic *et al*,2007). Different Gallic acid solutions were prepared having a concentration ranging from 50 μg/mL to 0 μg/mL. A volume of 2.5 mL of Folin-Ciocalteau reagent (diluted 10 times with water) and 2.0 mL of Na_2CO_3 (7.5% w/v) solution was added to 0.5 ml of Gallic acid solution. The mixture was incubated for 20 min at room temperature. After 20 min, the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa, a linear relationship was found which was used as a standard curve for the determination of the total phenolic content of the test samples. In 0.5 mL of extract solution (2 mg/mL), 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 mL of Na_2CO_3 (7.5% w/v) solution was added. The mixture was incubated for 20 min at room temperature. After 20 min, the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from Gallic acid solution with different concentration, the total phenolic content of the sample was determined. The phenolic contents of the sample were expressed as mg of (Gallic acid equivalent)/g of the extractive.

3.6.3 Determination of Flavonoid content

Reagents preparation

i. **10% $AlCl_3$ solution**

10 gm $AlCl_3$ was taken in a 100 ml volumetric flask and small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark adding required amount of distilled water.

ii. **1M potassium acetate solution**

9.8 gm of potassium acetate was taken in a 100ml volumetric flask and small amount of distilled water was added and dissolved in it. Then the final volume was made up to the mark by adding required amount of distilled water.

iii. **5% sodium nitrite solution**

5gm of $NaNO_2$ was taken in a volumetric flask and a small amount of distilled water was added in it and dissolved in it. Finally volume was up to the mark.

iv. **1M NaOH solution**

2g of NaOH was taken in a volumetric flask and a small amount of distilled water was added in it and dissolved in it. Finally volume was up to the mark.

v. **Standard rutin or quercetin solution**

Different rutin or quercetin solutions were prepared having a concentration ranging from 50µg/ml to 1000µg/ml for plotting standard curve.

Total Flavonoid Content

The total flavonoids content was estimated using the procedure described by Zhishen *et al.,* (1999). A total of 1ml of plant extracts were diluted with 200µL of distilled water separately followed by the addition of 150µL of sodium nitrite (5%) solution. The mixture was incubated for 5 minutes and then 150µL of aluminum chloride (10%) solution was added and allowed to stand for 6 min. Then 2 ml of sodium hydroxide (4%) solution was added and made up to 5mL with distilled water. The mixture was shaken well in a centrifuge at 4000rpm for 5 minutes and left it for 15 minutes at room temperature. The absorbance was measured at 510nm. Appearance of pink color showed the presence of flavonoids content. The total flavonoids content was expressed as rutin

equivalent mg RE or quercetin equivalent/g extract on a dry weight basis using the standard curve.

3.6.4 Determination of DPPH Radical Scavenging activity

Preparation of reagents

DPPH radical solution

0.3 mM DPPH radical solution was prepared by dissolving 5.91 mg of DPPH in 50 ml of methanol.

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Blois (1958) with slight modifications. 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of plant extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Corresponding blank sample were prepared and L- Ascorbic acid (1-100µg/ml) was used as reference standard. Mixer of 1 ml methanol and 1 ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm and after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula

Inhibition % = (Ac-As)/Ac×100.

Where Ac is the absorbance of the control as is the absorbance of the sample

3.6.5 Determination of Ferric Reducing Power

Preparation of reagents

Sodium phosphate buffer (pH 6.6) solution

Solution A = 0.2 M solution of Na_2PO_4 was made by dissolving 28.39g in 11 de-ionized water.

Solution B = 0.2 M solution of $NaH_2PO_4.2H_2O$ solution was made by dissolving 31.21g in 11 de-ionized water.

Then 18.75ml of solution A and 31.25ml of solution B were mixed together and diluted up to 100ml by deionized water.

1% potassium ferricyanide solution

1g of potassium ferricyanide was dissolved in 100ml of deionized water.

10% trichloroacetic acid

10g of trichloroacetic acid was dissolved in 100ml of deionized water.

0.1% ferric chloride solution

100g of ferric chloride solution was dissolved in 100ml of deionized water.

$FeSO_4.7H_2O$ stock solution

0.0556g $FeSO_4.7H_2O$ was dissolved in 200 ml distilled water making the solution of 1 mM. Then working solutions were prepared by serial dilution from (0.1- 1 mM)

Ferric Reducing Power

The reducing powder of the extract, which may serve as a reflection of its anti-oxidant activity was determined using a modified Fe^{+3} to Fe^{+2} reducing assay, whereby the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the sample. The presence of anti-oxidants in the sample causes the reducing of Fe^{+3} / Ferricyanide complex to the Fe^{+2} form (Do *et al*, 2014). This assay was slightly modified as the protocol reported by Oyaizu et al. In a test tube, 2.5ml (1 mg/ml) of the plant extract (or standard gallic acid solution) was taken and mixed with 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) and 2.5ml potassium ferricyanide solution (1%).The mixture was incubated at 50°C for 20 minutes. After incubation, 2.5ml trichloroacetic acid (10%) was added and the solution was centrifuged at 3000rpm for 10 minutes. Then 5/2.5 ml supernatant was mixed with 5/2.5 ml distilled water and 1/0.5 ml ferric chloride solution (0.1%) was added. Then blank had 2.5 ml methanol instead plant sample.The absorbance was measured at 700 nm. Standard curve was constructed using $FeSO_4.7H_2O$ in the concentrations ranged between (0.1-1.0) mM. Increased absorbance of the reaction mixture will indicated highest reducing power of samples.

Fig. 3.3 Ferric reducing power test

3.7 Physio-chemical analysis of water chestnut flour

3.7.1 Determination of moisture content of water chestnut flour:

Moisture content was determined by oven drying method (AOAC, 1995). An empty crucible washed, dried, cooled and weighed, then definite quantity (5gm) of water chestnut flour sample was taken in the crucible and was weighed. The crucible was placed in the oven and was dried at a temperature of 105° C for 24 hours. After drying the crucible was removed from the oven and cooled in desiccators and then weighed. Crucible with sample was again placed in the oven, dried for 30 minutes, taken out from the oven, cooled in desiccators and weighed. Drying, cooling and weighing were repeated until two consecutive weights were same, for accuracy at least 3 samples were dried in the oven and average moisture content was then calculated as follows:

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

3.7.2 Determination of ash content of water chestnut flour:

Ash content of a food stuff represents inorganic residue remaining after destruction of organic matter.The total ash content in sample was determined by AOAC, 2000 method. The oven dried flour sample was taken in a crucible and left inside muffle furnace at 550°C for 6 hrs.The muffle furnace was turned off and waited till the temperature dropped at least 250°C,carefully opened the door and transferred the crucible to a desiccator to avoid losing ash. After cooling the weight of crucible was recorded.The difference between the weight of oven dried matter and final weight represented the ash

content of flour, which was expressed in percentage. It was calculated by using the following formula:

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Initial weight of the sample}} \times 100$$

3.7.3 Determination of Fiber content of water chestnut flour:

Dietary fiber can be defined as sum of polysaccharides and lignin that are not digested by human digestive enzymes. The major components of dietary fiber are cellulose,non-cellulose such as hemicelluloses and pectin, lignin, and hydrocolloids.This method gives the fiber content of the sample after it has been digested in sulphuric acid and sodium hydroxide solutions and the residue calcinated. The difference in weight after calcination indicates the quantity of fiber present. The fiber content of prepared cake, sweet biscuit and plain bread was determined by acid-alkaline digestion method. (Aisyah Bujang, 2011).

Reagents:

- ✓ Sulphuric acid solution (1.25% v/v), 200ml
- ✓ Sodium hydroxide solution (1.25% v/v), 200ml
- ✓ Ethyl alcohol at 95% (v/v).

Materials and Equipments:

- ✓ 1000 ml Beaker (3)
- ✓ Auto heater
- ✓ Filtration crucible.
- ✓ Thin cloth
- ✓ Hot air dryer.
- ✓ Muffle furnace.
- ✓ Water chestnut flour

Procedure:

Weighted out 15 g of water chestnut flour.Then add 200 ml sulphuric acid (H 2 SO 4) solution into the sample. Then placed the beaker on an automatic heater and turned on the heater. Boiled this mixture for 30minutes with continuous stirring. During boiling, sulphuric acid digested the dissolved component of the sample. After boiling, the

solution was filtrated by using a clean and thin cloth. Then the solid residue was collected from the cloth very carefully and taken it in another beaker. Then added 200 ml NaOH (1.25%) into it and the solution was boiled for 30 min with continuous stirring. After completing the boiling, the solution was filtrated again by cloth and collected the solid residue in a crucible. Washed the residue with boiling water, HCI solution and boiling water respectively. Placed the crucible in hot air oven and set at 105°C for 12 hours and cooled in dryer. Quickly weighed the crucible with the residue and placed in the muffle furnace at 550°C for 3 hours. After that the crucible was brought out from the dryer and weighed them again.

Formula for estimation of percent fiber content:

$$\% \text{ Fiber content} = \frac{\text{Weight of crucible with ash} - \text{weight of ash found}}{\text{weight of the sample}} \times 100$$

Sample boiled with H_2SO_4

Filter the solution through cloth

Separated liquid after filtration

Ash content after drying

3.7.4 Determination of Protein content of water chestnut flour

The Protein content of water chestnut flour was determined by Kjeldahl method (Kjeldahl J., 1883).

Principle of Kjeldahl method

The Kjeldahl procedure measures the nitrogen content of a sample. The protein content then, can be calculated assuming a ratio of protein to nitrogen for the specific food being analyzed. The Kjeldahl procedure can be basically divided into three parts:

(1) Digestion
(2) Distillation
(3) Titration

In the digestion step, organic nitrogen is converted to an ammonium in the presence of a catalyst at approximately 370°C. In the distillation step, the digested sample is made alkaline with NaOH and the nitrogen is distilled of as NH_3. This NH_3 is "trapped" in a boric acid solution. The amount of ammonia nitrogen in this solution is quantified by titration with a standard HCl solution. A reagent blank is carried through the analysis and the volume of HCl titrant required for this blank is subtracted from each determination.

Reagents need for protein estimation

1. Potassium sulfate, K_2SO_4
2. Copper sulfate
3. Concentrated H_2SO_4 (98% v/v, already prepared)
4. 40%(w/v) NaOH for each digestion tube(already prepared)
5. 4% Boric acid, H_3BO_3 (already prepared)

Equipments need for protein estimation

1. Erlenmeyer flask (digestion flask)
2. Digestion tube
3. Burette

Procedure: (A.M.Y. Jaber, N.A. Mehanna, S.M. Sultan, 2009)

A. Digestion of the sample:

1 gm water chestnut flour was taken in digestion flasks. Then added the followings into them:

K_2SO_4(5gm), Copper sulfate (1gm), Concentrated H_2SO_4(98%, 25 ml)

Then the flasks were taken on automatic heating chamber and heated them until fresh residue was found.

Digestion is the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution. The general equation for the digestion of an organic sample is shown below:

Protein + $H_2SO_4 \rightarrow (NH_4)_2SO_4$ (aq) + CO_2(g) + SO_2(g) + H_2O(g)

Sulfuric acid has been used alone for the digestion of organic samples. The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO_2 evolves.

B. Distillation:

i. Placed the digested samples in digestion tubes to the distilling unit and added 100 mL of 40% (w/v) NaOH.

ii. The sample was being distilled until 100 mL of distillate were collected in 25.0 mL of 4.0 % (w/v) boric acid.

The majority of the NH 3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture

and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen.

C. Titration:

Added 2-3 drops indicator to the distillation flask and titrate it with 0.2 N HCl (in Burette). At this direct titration, end point indicate the ammonia present in the distillate with a color change (end point pink color shown). Then recorded volume of HCl titrant used and allowed for calculation of percent protein content.

Formula for estimation of percent protein content:

$$\% \text{ Protein content} = \frac{\text{Nitrogen factor } (1.4007) \times \text{ml of standard acid(HCl) need for titration} \times \text{normality of HCl} \times \text{protein factor for specific sample}}{\text{Weight of sample}}$$

3.7.5 Determination of fat content of water chestnut flour

Fat content of water chestnut flour was determined by Solvent extraction (Soxhlet extraction) method. (Soxhlet, F;1879)

Principle of solvent extraction (Soxhlet extraction) Method

Lipid in food present in various forms like mono glycerides, di-glycerides, tri-glycerides and sterol and free fatty acid and phospholipid and carotenoids and fat-soluble vitamins. Lipid is soluble in organic solvent and insoluble in water, because of this, organic solvents like hexane, petroleum ether have the ability to solubilize fat and fat is extracted from food in combination with the solvent. Later the fat is collected by evaporating the solvent. Almost all the solvent is distilled off and can be reused (Julius B, 1910).

Material and equipment:

- ✓ Weighing balance
- ✓ Soxhlet apparatus
- ✓ Drying oven
- ✓ Thimble
- ✓ Heating mantle
- ✓ Glass rod
- ✓ Desiccator with silica gel

- ✓ Cotton plugs
- ✓ Water chestnut flour

Reagent:

- ✓ Petroleum ether (Boiling temperature 60°-80°C)

Procedure:

First of all, all the glass apparatus were rinsed by petroleum ether and dried them in the oven at 102°C for 30 min and cooled in a desiccator. Then 5 gm of water chestnut flour was placed in a weighed thimble. The thimble was placed in the soxhlet extractor. After that, a 150 ml cleaned round bottom flask was filled with 90 ml petroleum ether. The extraction unit was assembled over an electric heating mantle and allowed the petroleum ether to boil. The extraction process was continued almost 6 hours.

The extraction unit was removed from the heat source and detached the extractor and condenser. Then the collected solvent from the flask after extraction was taken in a crucible. The crucible was placed in the hot air oven and allowed it for drying at 105°C for 6 hours. After completing the drying, the crucible was placed in the desicator for cooling. Then weighed the crucible and recorded the value.

Formula for estimation of percent fat content:

$$\% \text{ Crude fat content} = \frac{\text{Weight of flask with dried fat} - \text{Weight of flask}}{\text{Initial weight of taken sample}} \times 100.$$

3.7.6 Determination of available carbohydrate content of water chestnut flour

Available carbohydrate represents that fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism. Available carbohydrate can be arrived at in two different ways: it can be estimated by difference or analyzed directly. In this experiment the available carbohydrate content of water chestnut flour was determined by difference method (AOAC, 2000).

Formula for estimation of available carbohydrate content by difference method:

Available carbohydrate % = 100 - (Protein% + Fat% + Moisture% + Ash% + Dietary fiber%) in 100 gm of food.

3.8 Sensory analysis of water chestnut kernel and cookies

The sensory quality of prepared cookies were determined by Hedonic scale method (Peryam, D.R. and Girardot; 1955).

The most widely used scale for measuring food acceptability is the 9-point hedonic scale (Peryam, D.R. and Girardot; 1955).

The verbal anchors of the scale were selected so that the psychological distance between successive scale points is approximately equal. This equal-interval property helps justify the practice of analyzing the responses by assigning successive integer values (1, 2, 3, … up to 9) to the scale points and testing differences in average acceptability using parametric statistics. The reliability, validity and discriminative ability of the scale was proved in food acceptance tests with soldiers in the field and in the laboratory.

3.8.1 Panel member selection process

Selection of panelists, twenty people were selected from a pool of volunteers comprising professors, lecturers, and students of food and process engineering, Hajee Mohammad Danesh Science and Technology University. The panelists were selected after an oral interview conducted on the basis of a criteria checklist that included:

Good health, nonsmoker, non allergic to wheat flour, willingness to participate, and passion/likeness for the consumption of cake, biscuit and bread.

3.8.2 Sensory evaluation process

First of all a hedonic test rating chart was given to the panelist. We setup some rating in those chart according to their preference. We conduct our test according to some sensory characteristics of the product, that's are given below:

Parameter tested in evaluation process:

- ✓ Color
- ✓ Texture
- ✓ Flavor
- ✓ Taste
- ✓ Overall acceptability

Rating (hedonic scale) set up in the chart: (Peryam, D.R. and Pilgrim, F.J. 1955)

Table 3.2 Hedonic scale rating chart

Level of preference	Rating
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

CHAPTER 4

RESULTS AND DISCUSSIONS

The current study on "Physical properties, proximate composition and anti-oxidant properties of water chestnut (*Trapa bispinosa* Roxb.) flour and quality evaluation of cookies". The material and methods used for the study are described in this section.

4.1 Result of physical and functional properties of water chestnut flour and its cookies:

4.1.1 pH and Gluten content:

After performed gluten presence test no sticky substance (gluten chain) was found in the dough. So according to the experiment, it was found that there was no gluten present in water chestnut flour. The water chestnut flour totally gluten free was reported by Joly S., 2007. The result of pH reported by this study was 5.11 which was closely related to the value 5.88 reported by Omar F.M *et al.(*2012).

4.1.2 Bulk density

Bulk density (loose) and bulk density (packed) were given in Table 4.4. Bulk density (loose) of WCF was 0.52g/ml where as bulk density (packed) was found to be 0.79g/ml. Bulk density depends on the particle size of the samples. It is a measure of heaviness of a flour sample. It is important for determining packaging requirements Singh *et al.* (2011); material handling and application in wet processing in the food industry Yellavila *et al*. (2015). Increase in bulk density is desirable since it offers greater packaging advantage by allowing packaging of greater quantity within constant volume.

Table 4.1 Physical properties of Wheat Flour and Water Chestnut Flour.

Sample	Loose bulk density (g/ml)	Packed bulk density (g/ml)	Water absorption capacity (%)	Oil absorption capacity (%)	Swelling power (g/g)	Solubility (g/g)
Water chestnut flour	0.52±0.01	0.77±0.01	110±0.577	76±0.43	7.9±0.23	0.2±0.05

n=3; means ± standard deviation.

4.1.3 Water absorption capacity (WAC)

Water absorption capacity (WAC) of flour plays an important role in food preparation due to its influence on other functional and sensory properties. WAC is the ability of a product to associate with water under limiting conditions. WAC of WCF was 110% (Table 4.1). Mir *et al.* (2015) reported lesser 102.3% WAC of WCF than the results reported here. The discrepancy could be due to and the method used. However, similar results 112% were found by Singh *et al.* (2011) and attributed WAC of WCF to its lower protein and lipid fraction. This suggests that higher WAC of food material is advantageous for baked products where hydration improves dough handling, a preferred characteristic. Okpala *et al.* (2013) reported high WAC of the studied composite flours and suggested it to be useful for bakery products as this could prevent staling by reducing moisture loss.

4.1.4 Oil absorption capacity (OAC)

Oil absorption capacity (OAC) is the ability of the flour to absorb oil, which is important as oil acts as a flavor retainer and improves mouth feel. The result of OAC of WCF was 76% (Table 4.1).The result was higher than Bala *et al.* (2015). Bala *et al* (2015) reported that the OAC was 62.35%. Shafi M. *et al* (2016) reported the OAC was 80%.

4.1.5 Swelling power and solubility

The swelling power of WCF was found to be 7.95%. Swelling capacity is related to protein and starch content of the flour. High protein content in flour may limit the access of starch granule to water and thus swelling power of starch is restricted (David *et al*. 2015). High swelling power of WCF could be attributed to its low protein and high carbohydrate content. The extent of swelling of the flour also depends on the temperature, availability of water, type of starch, extent of starch damage and other carbohydrates (such as pectins, hemicelluloses and cellulose) and protein. Solubility of WCF was found to be 0.2 g/g. Factors that may influence starch solubility were source, swelling power, inter-associative forces within the amorphous and crystalline domains, and presence of other components such as phosphorous.

4.2 Proximate composition of flours and cookies of Water chestnut

The proximate composition of Water chestnut flour and its cookies is presented in Table 4.2.

Table 4.2 The proximate composition of Water chestnut flour and its cookies made of 100% water chestnut flour.

Compositions	Water chestnut flour	Cookies
Moisture	9.77±0.11	3.93±0.15
Protein	4.33±0.05	3.03±0.23
Fat	0.33±0.03	15.3±0.60
Carbohydrate	81.59±0.17	73.07±0.44
Crude Fiber	1.51±0.02	1.9±0.02
Ash	2.47±0.06	2.7±0.11

n=3; means ± standard deviation.

4.2.1 Moisture content

The moisture content of the WCF was 9.77% (Table 4.2). The result was higher than the result reported by Alfasane *et al*. (2011) (7.30)% where-as closely related to a recent report by Bala *et al.*(2015) who demonstrated that water chestnuts (*Trapa bispinosa* Roxb.) contained moisture 9.0%. The result of water chestnut cookies was 3.93% (Table 4.2).

4.2.2 Protein content

The protein content of WCF and cookies was found to be 4.33% and 3.03% (Table 4.2) respectively. The value of WCF was lower than the result reported by Ahmed *et al*. (2016) (8.4%) where-as Bala *et al*. (2015) and Singh *et al*. (2011) reported fairly similar protein content of WCF (3.1%). Variations in protein content can be attributed to different environmental conditions, genotype and analytical methods. In addition, protein content was sensitive to rainfall, light intensity, length of growing season, day duration, temperature and agronomic practices (Bampidis *et al.*, 2011).

4.2.3 Fat content

The fat content of WCF and cookies were 0.33% and 15.3% (Table 4.2). Here Mean ± standard deviation values (n=3) was calculated by MS Excel V2013. The fat content 0.52% for water chestnut flour was reported by Shafi M. *et al*. (2016). But Bala *et al*. (2015) reported the value of fat was 0.93%.

4.2.4 Ash content

The ash content of WCF and cookies were 2.47% and 2.7% (Table 4.2). Bala *et al*. (2015) reported the ash content was 2.42% similar value. Shafi M. *et al*. (2016) also reported the value of ash was 2.32%.

4.2.5 Total carbohydrate content

The total carbohydrate content of WCF and cookies were 81.59% and 73.07% (Table 4.2) respectively. That result was agreed with the report of Bala *et al*.(2015) (83%). Shafi M. *et al.* (2016) reported the moisture content was 81%.

4.2.6 Crude fiber

The result of crude fiber of WCF and cookies were 1.51% and 1.9% (Table 4.2) respectively. That result was less than the report by Alfasane *et al.* (2011) (2.05%). But Ahmed *et al.* (2016) also reported similar crude fiber content of WCF.

4.3 Antioxidant analysis of flour and cookies

4.3.1 DPPH scavenging activity

DPPH scavenging activity of WCF and cookies is shown in Table 4.3. WCF showed DPPH scavenging activity was 52.38%. Polyphenolic compounds present in water chestnut can be responsible for higher DPPH scavenging activity of WCF. Baking resulted in an increase in the DPPH scavenging activity of cookies in comparison to flour. Increase in DPPH scavenging activity due to baking has already been reported (Jan *et al*. 2015) and has been attributed to melanoidins formed during processing (Baba 2015; Nisar *et al*. 2015). However, baking resulted in greater increase in scavenging activity of WC cookies (73.15%). However, it should not be ruled out that antioxidant activity may not only be affected by temperature but also exhibits synergism (Brewer 2011).

Table 4.3 Effect of baking on antioxidant properties of wheat and cookies made of 100% water chestnut flour.

Sample	DPPH (% inhibition)	FRP (mg/g)	TFC (mg/g)	TPC (mg/g)
WCF	52.38±5.77	137.51±5.2	0.21±0.11	4.27±0.20
Cookies	73.15±4.49	116.67±7.56	1.51±0.18	3.5±0.23

WCF= Water chestnut flour.
DPPH= deoxyribonucleic acid. FRP=Ferric Reducing Power.
TFC=Total Flavanoid Content. TPC=Total Phenolic Compound.

4.3.2 FRP

FRP values showed a similar trend as seen in DPPH scavenging activity. Baking increased the FRP values of flour. An increase in the FRP values after baking was previously reported in buckwheat Jan *et al.*(2015) amaranth and quinoa flours Chlopicka *et al.*(2012). However, greater increase was seen in WC cookies. Jan *et al.* (2015) also reported a greater increase in FRP value of buckwheat cookies than those of wheat flour. Water chestnut like buckwheat is reported to contain high levels of quercetin that show antioxidant activity even at 150 °C Elhamirad and Zamanipoor (2012) which might be responsible for higher activity of water chestnut cookies.

4.3.3 Total Phenolic Content (TPC)

TPC of WCF and cookies is shown in Table 4.3. WCF showed TPC content was 4.27 mg/g. Baking decreased the TPC content in WCF during baking. In this study, a decrease of 36% was seen in TPC of WC cookies. Cookies made from 100% WCF showed the decrease in TPC was (20.70%). It is worth mentioning here that WC has previously been reported to contain both the parent phenolic acid derivatives viz. hydrocinammic acid and hydroxybenzoic acid derivatives in the form of ferulic acid, p-coumaric acid and gallic acid respectively Yu *et al.* (2013).

4.3.4 Total Flavonoid Content (TFC)

TFC of WCF as well as their cookies is shown in Table 4.3. WCF had flavonoid content about (1.92 g QE/1000 g). TFC of WCF cookies (1.51 mg/g) decreased significantly

during baking. However, this can be attributed to the type of flavonoids present in WCF. One of the major flavonoids present in WCF was quercetin (Chiang *et al.* 2008). Elhamirad and Zamanipoor (2012) have reported Quercetin to have higher values of standard coefficient (Q), a measure of antioxidant activity at higher temperatures (140, 160, 180 °C). Thus, it can be suggested that an improved retention of antioxidant activity in water chestnut at higher temperatures can be due to hydroxylation of quercetin (B-ring). Elhamirad and Zamanipoor (2012) gave similar explanation for thermal stability of flavonoids in sheep tallow olien frying. Better retention of antioxidant activity due to hydroxylation of flavonoids is indirectly favored by the fact that WCF has greater percentage of flavonoids than wheat flour.

4.4 Sensory evaluation

Sensory attributes like color, flavor, texture, taste and overall acceptability of prepared cookies were evaluated by 20 semi trained panelists. They given score according to hedonic scale rating (Table 3.2). Mean score for color of cookies made from different ration of water chestnut flour and wheat flour were presented in table 4.4. The result showed that the sample 123 (20% WCF and 80% WF) was most accepted to the panelist. The overall acceptability was 8.45 out of 9. It was accepted as same as control. The bar diagram of overall acceptability of different cookie's samples were given in fig. 4.1.

Table 4.4 Mean scores of cookies made from blending of WCF and WF.

Sample	Color	Flavor	Texture	Taste	Overall acceptability
101	8.45	7.7	8.3	7.15	7.85
123	8.45	7.95	7.65	7.25	7.85
231	8.1	7.7	7.3	7.2	7.45
321	7.85	7.7	7.25	7.35	7.45
456	7.7	7.65	6.9	7.45	7.5
564	7.25	6.95	6.75	6.45	6.75

Sample 101 (Control)= 100% Wheat Flour (WF) Sample 123= 80% WF and 20% WCF
Sample 231=60% WF and 40% WCF Sample 321= 40% WF and 60% WCF
Sample 456= 20% WF and 80% WCF Sample 564= 100% WCF.

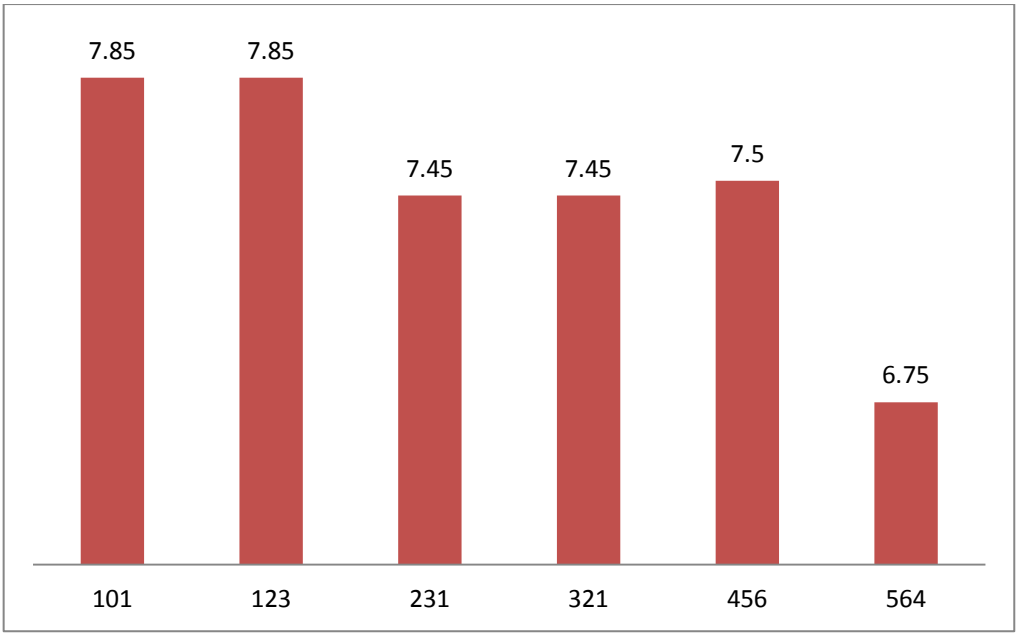


Fig 4.1 Diagram of overall acceptability of cookies

CHAPTER 5

SUMMERY AND CONCLUSION

Gluten-free cookies were prepared from water chestnut flour subjected to nutritional assessment and sensory analysis. Wheat flour can be completely replaced by water chestnut flour for production of gluten free cookies with improved taste and flavor. However, some modification such as incorporation of skimmed milk powder to improve texture and color of cookies is recommended. Sensory analysis of water chestnut cookies revealed that the cookies prepared from 20% WCF with 80% Wheat flour were best accepted to the sensory panel in terms of physical appearance, crust and crumb color, flavor, texture and taste. Water chestnut flour shows better retention of antioxidant properties than blending wheat flour during baking. Further research regarding antioxidant survival during processing and product development using water chestnut flour is strongly recommended and also need a research on the utilization of this underutilized crop for preparation of various bakery products which will help for consumption of people who cannot tolerate gluten content and uplift the socioeconomic conditions of people associated with this trade in Bangladesh.

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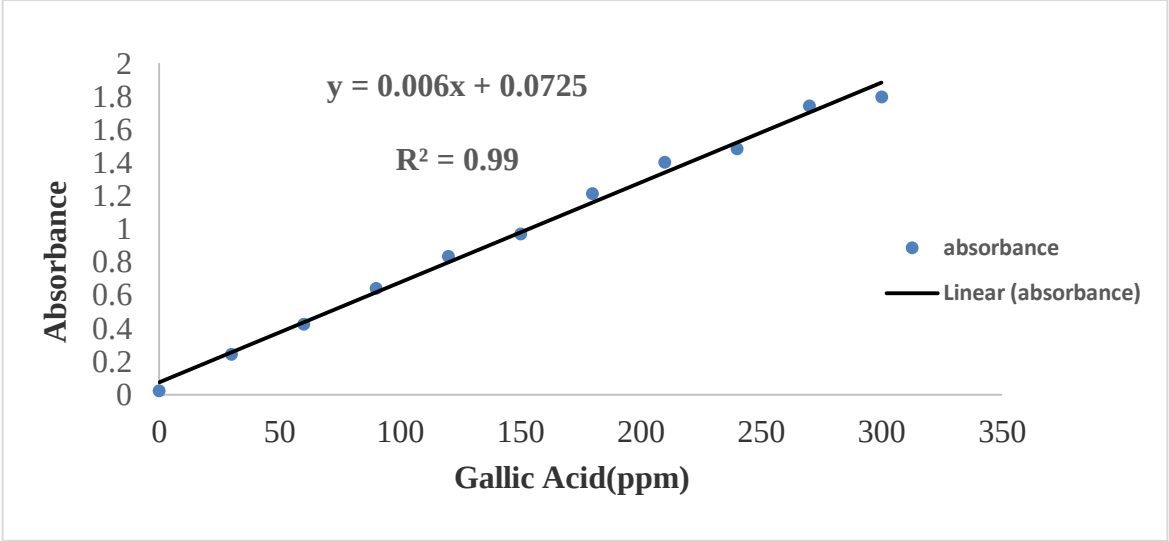
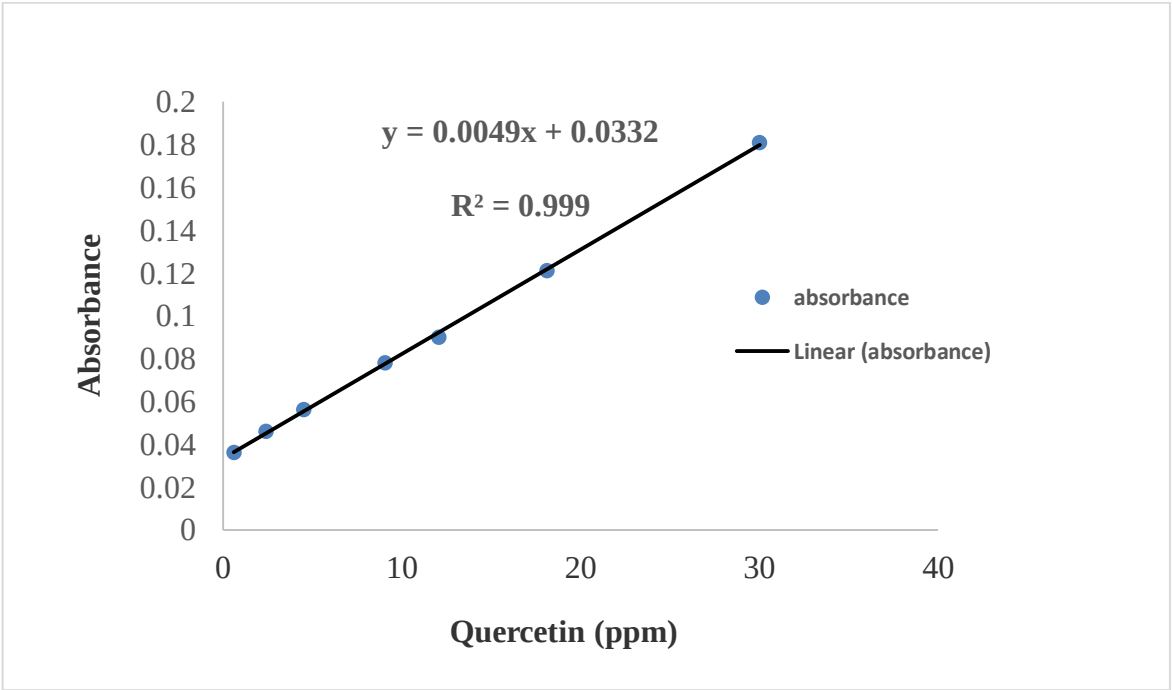
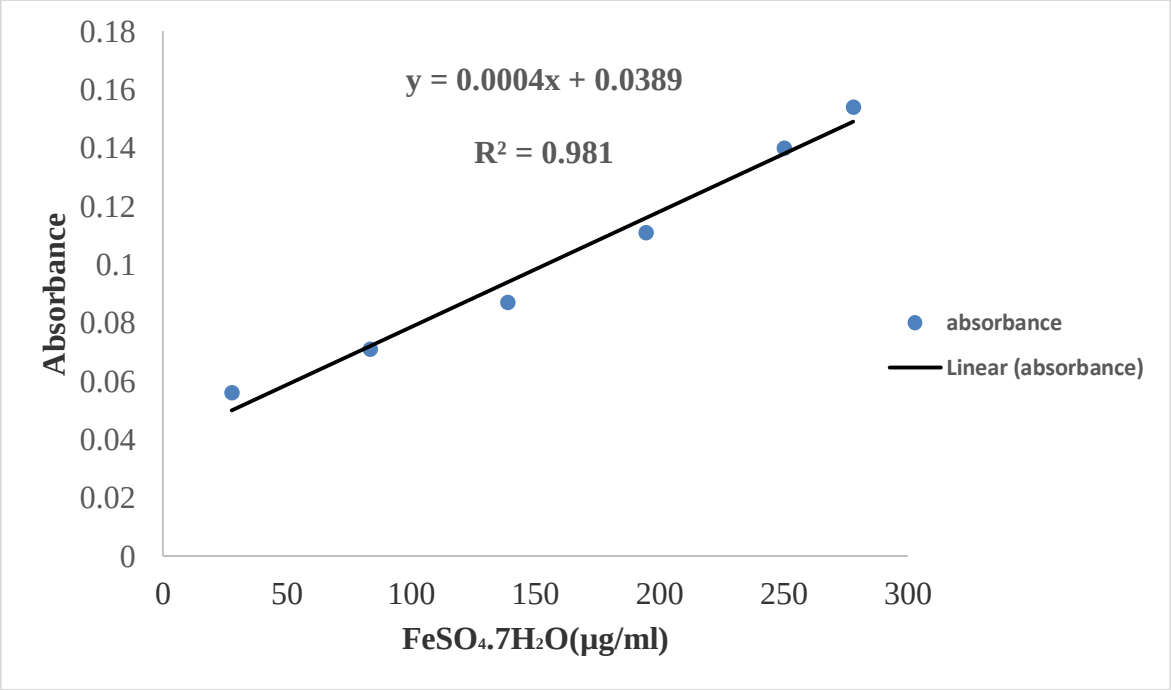
Appendix I: Standard Curve for Total Phenol



Appendix II: Standard Curve for Total Flavonoids



Appendix III: Standard Curve for FRAP



Appendix IV Descriptive Analysis for Sensory Evaluation.

Sample	Color	Flavor	Texture	Taste	Overall acceptability
101	8.45±0.60	7.7±0.73	8.3±0.66	7.15±0.67	7.85±0.75
123	8.45±0.60	7.95±0.76	7.65±0.67	7.25±0.79	7.85±58
231	8.1±0.72	7.7±0.65	7.3±0.57	7.2±1.06	7.45±0.60
321	7.85±0.67	7.7±0.73	7.25±0.78	7.35±1.18	7.45±0.94
456	7.7±0.73	7.65±0.67	6.9±1.02	7.45±1.05	7.5±0.83
564	7.25±0.64	6.95±0.51	6.75±0.78	6.45±0.94	6.75±0.72