DEVELOPMENT OF MIXED FRUIT TOFFEE AND ITS NUTRITIONAL QUALITY, ANTIOXIDANT ACTIVITY AND MICROBIAL LOAD EVALUATION

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Roll No. 0118/18

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

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DECEMBER 2019

Dedicated To

My Beloved Family

&

Honorable Teachers

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List of Abbreviations

%	Percentage
º C	Degree Celsius
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agriculture Research Institute
BBS	Bangladesh Bureau of Statistics
DPPH	2,2-diphenyl-hydrazyl-hydrate
et al	Et alii/ et alia/ et aliae
g	Gram
Kcal	Kilo calorie
Kg	Kilogram
mg	Milligram
SMP	Skimmed Milk Powder
SPSS	Statistical Package for Social Science
Tk	Taka
TE	Trolox Equivalent
TSS	Total Soluble Solids
TVC	Total Viable Count

Abstract

The present study was carried out to develop a technology for preparation of mixed fruit toffee from guava pulp and Indian olive pulp. Guava belongs to the major fruit groups of Bangladesh and Indian olive exists in the minor fruit groups. In this research work, the chemical composition, mineral contents, antioxidant capacity, microbial status and sensory properties of the mixed fruit toffee were determined. The investigation was taken place by preparing toffee in addition of sugar, fat, glucose, Skimmed milk powder and salt in both pulp. Toffees was made from four formulations including 100:00, 80:20, 70: 30, 60:40 ratios of Guava : Indian olive pulp. The combination of guava: Indian olive (100:00) was found best than other combinations in respect to organoleptic properties and the formulation of Guava: Indian olive (60:40) was superior in nutritional quality. The toffees prepared were wrapped in metallic coated polythene wrapper, packed in 200 gauge polythene bags and stored at ambient (30 ±2ºC) temperature. The mean score of fresh toffees with the preferable formulation for color and appearance was 7.67 and 7.66 respectively, texture 7.73, flavor 8.07, taste 8.20 and overall acceptability 7.93 on 9 point hedonic scales. The carbohydrate, fat, protein, ash, fiber were determined at the range of 57% to 67.43%, 3.59% to 4.88%, 4.6% to 5.3%, 1.41 to 1.68% and 4.24 % to 9.26% respectively. Energy content was found ranging from 299-341 kcal/100g. Vitamin C content in mixed fruit toffee was estimated ranging (50- 91) mg/100gm and was highest amount in the formulation of guava: Indian olive (60:40). The cost of mixed fruit toffee was ranged from 4.89 to 5.01 taka for various combinations of ingredients. Antioxidant capacity was higher in formulation with guava: Indian olive (70:30) which showed antioxidant properties of mean 30.9 (mg TE/100 g). Microbial status showed a great results with total viable count with acceptable limit and absence of specified bacteria *E.coli* and Salmonella and fungal activity was not seen after 1 month of storage at ambient temperature.

Keywords: Guava, Indian olive, Toffee, Mixed fruit toffee, Sensory properties

Chapter-1: Introduction

The Asian area is wealthy in wide variety of tropical fruit species, especially in South and Southeast Asia. Fruits are significant for the people in the area, as wellsprings of supplemental nourishment, nutritionally balanced, and assist to shield from sickness. Whereas some species possess with medicinal values, while others are utilized for timber, fuel wood and domesticated animals feed. The South Asian locale containing India, Pakistan, Bangladesh, Nepal, Sri Lanka, Bhutan and the Maldives has a wide scope of climatic conditions with the height and agro-environment appropriate for a wide decent variety of tropical fruits. This area is the focal point of starting point of around 50 species of fruits. Around 20 of these are significant and are cultivated in a various countries. The significant fruits cultivated in these nations are banana, mango, citrus, pineapple and papaya (Chandha, 1989).

In excess of 90 vegetables and 60 fruits are cultivated in Bangladesh, with great provincial variety in the degree of development. May, June and July are particularly known as fruit celebration months in Bangladesh when nearly all the major and minor fruits are accessible and matured. The major fruits are the fruits which are mostly cultivated such as mango, jackfruit, pineapple, banana, litchi, guava, papaya, melon, watermelon and plum. On the other hand, the minor fruits are those which are less grown and these are famous among them: Black berry (kalo jam), Tamarind (tetul), Palmyra palm (tal), Monkey jack (dewa), Indian olive (jalpai), Carambola (kamranga), Star apple (jamrul), Mangosteen (kaw), Burmese grape (latkan), Velvet apple (bilati talk), Wood apple (kathbel), Indian apple (bel), Custard apple (ata), and Indian Goose berry (amlaki)(Hoque et al., 2009).

Guava (*Psidium guajava*) pertains to family Myrtaceae that comprise of around 100 species of tropical shrubs and small trees. Guavas are predominantly grown in tropical and sub-tropical nations. It is highly produced in Bangladesh and available throughout the country with a reasonable price. Bangladesh is eighth most elevated guava creating nations. In Bangladesh the yearly cultivation is 1,048,850 MT in 2017-18 (Sakib et al., 2018). Common name of guava utilized in Bangladesh is "Piara". The

fruit contains around multiple times the measure of nutrient C as present in orange. Guava contains the two carotenoids and polyphenols like leucocyanidin, guaijaverin, gallocatechin, and the significant classes of antioxidants which give those high value of antioxidant among plant foods. Guava is additionally discovered viable against malignant growth, bacterial diseases, irritation and agony. It is preferably eaten in raw form. A wide assortment of significant worth included items can be set up from this organic product including drinks, jam, jelly, toffee and cheese. It is considered as "magical" fruit due to its variety of nutrients and therapeutic uses. It has a rich ethno-therapeutic history. Various pieces of the plant are utilized in different indigenous frameworks of medication, basically for the treatment of gastrointestinal issue (Chauhcin and Cahoon, 1987; Rao and Mukherjee, 1989; Haag et al., 1990). The guava fruit is an excellent source of vitamin C upto 2000 mg/100 gm fruit. Fruit is abundant in dietary fiber (from 5-7%), vitamin A, pectin, phosphorous, calcium and potassium (Khapre, 2010).

The strong aroma of guava fruits is attributed to carbonyl compounds. Guava pulp had very strong flavor. Therefore, it will be very wrathful to mix guava pulp with other fruit pulp having less flavour to form combination of both to yield good quality processed fruit product (Chavan et al., 2016).

Indian olive has a place with the minor fruits gathering of Bangladesh which is named experimentally "*Elaeocarpus floribundus*" with Elaeocarpaceae family. Elaeocarpus is a class of 350 plants species with a wide circulation in Madagascar, India, Southeast Asia, Malaysia, southern China and Japan just as Australia and New Zealand, Fiji and Hawaii in the east (Burkill et al., 1966) In Bangladesh, it is regularly known as “jalpai”. Jalpai is a medium to tall tree; their leaves are simple, green in shading, regularly normal presences of certain leaves which are red or orange in shading. Blossoms show up during April to May and greenish natural products develop for gathering in August to October. Jalpai are greenish, single seeded, drupe and consumable bit is mesocarp around the seeds. This acidic develop and

juvenile jalpai is mostly utilized for processing of chutney, pickles. The therapeutic properties of various types of Elaeocarpus and the Phenolic substance, cancer prevention agent and cytotoxic exercises of *Elaeocarpus floribundus* was accounted for by the few researchers (Dhadich et al, 2013; Utami et al, 2013). They are acrid in taste and essentially contain nutrient C. They are wealthy in calcium and iron. They have some therapeutic qualities like distrate, torment and cooling impact. Indian olives are used for arrangement of chutney, making pickles and other culinary purposes (Moshiur and Jillur, 2014).

Toffees are the sugar confectionary containing sugar, milk solids and margarine or vegetable fat as the significant fixings. Because of their taste and flavor, they appreciate wide prominence. This prominence could be put to appropriate use by expanding the nutritive estimation of toffees as far as proteins, minerals and nutrients. In mixed fruit toffee, the mixing of pulpy fruits were done which contributes towards improving the protein and mineral substance of the toffees. Furthermore an assortment of flavors can be gotten with fresh fruit pulp in toffee.

In this study, mixed fruit toffee were prepared from guava and Indian olive. In the fruit mixture, guava was used as base in addition of Indian olive. The study was designed to prepare the toffee in four formulations (100% guava pulp + 00% Indian olive pulp, 80% guava pulp + 20% Indian olive pulp, 70% guava pulp + 30 % Indian olive, 60 % guava pulp + 40% Indian olive). Guava used as base because it is more available and preferable than Indian olive to most of the people of Bangladesh. On the other hand, Indian olive is a sour fruit and not available in all year round. Furthermore, Indian olive percentage was lower in the four formulations as guava tastes sweet and there was a need of little bit sour taste in the mixed fruit toffee. Fruits are rich in mineral and fiber so addition of fruit can be a significant implication for developing a product. Guava and Indian olive also a great source of vitamin C which provide a wide variety of nutrition in the toffee. Furthermore, both the fruits are abundant in micronutrients and wholesome food for human body that need to be utilized to develop a processed food.

Sometimes people don’t want to consume whole fruits and for them this product can be a good choice to get nourishment.

The aims and objectives of this study were:

1. To develop a product, mixed fruit toffee from guava and Indian olive
2. To determine the nutritional quality of the developed product
3. To evaluate antioxidant activity of the developed product
4. To analyse major mineral content, vitamin C content, microbial load and sensory evaluation of the final product
5. To analyse cost for the production of the product

Chapter-2: Review of Literature

2.1 Guava fruit in brief

Guava belongs to the family myrtle (Myrtaceae) family containing about 100 species of the shrubs of tropical and the small trees. It is native to Mexico, northern South Africa, Central America, parts of Caribbean and North Africa. Guavas are now produced naturalized throughout tropics and are also cultivated in part of subtropical regions. Guava is known as different names in different regions of the world. In Bengali, Brazil, Arabic, Cambodia, English, Chinese, Germany, French, India, Thailand, Spanish, Portuguese and Philippines guava fruit is known as Piara, Araca, Guwâfah, Trapaeksruk, Apple guava, Fan shiliu, Guavenbaum, Gouyave, Amarood, Farang, Guayaba, Goiaba, Bayabas respectively (Jimmy Wales, 2008).

2.2 Worldwide cultivation and distribution of guava

Now, guava can be seen growing in not less than fifty countries in the tropics, subtropics and some parts of Mediterranean areas. In Europe, this fruit is produced in Spain, Israel, Portugal, and southern Part of France. In the United States, this fruit can be seen in California, Hawaii and Florida. Also in the native habitat of northern part and central part of South America. The main producers around the world are Mexico, Brazil and India. Other countries which are leading namely Jamaica, Columbia, the USA (major- Florida, Hawaii), South Africa, Kenya, Egypt, Cuba and the Philippines. In India, 200,000 metric tons guava were grown. In Bangladesh, Mexico, Cuba, Egypt, South Africa 146,077 metric tons, 175,000 metric tons, 90,714 metric tons, 34,000 metric tons, 13,000 metric tons were grown respectively. It is palpable that the cultivation of guava in our country is increasing year to year. It can be said that the maximum growing of guava is in India and minimum producing country is South Africa whereas Bangladesh has stood by the third rank in the world for the cultivation of guava (Hossen, 2013).

2.3 Food value of guava fruit

Guava contains rich amount of tannins, flavonoids, phenols, essential oils, saponins, trierpenes, carotenoids, fiber, fatty acids, vitamins, lectins etc. High amount of vitamin C presents in guava than citrus (80mg/100 g fruit). It also contains high amount of vitamin A and pectin – a dietary fiber. It has broad spectrum of phytochemicals such as vitamins, polysaccharides and essential oil. Guava is renowned as a booster for

vision and health which possesses a good source of vitamin, mineral, protein, lipidetc. (Joseph and Priya, 2011). The food value of 100g guava is tabulated below:

Table 2.1: Food value of guava (Kamanth et al., 2008)

Nutrient	**Content**
Calorie	77-86 g
Moisture	2.8-5.50 g
Fat	0.43-.7 mg
Fiber	0.90-1 g
Protein	0.1-0.50
Carbohydrate	9.10-17 mg
Ash	9.50-10 mg
Calcium	17.80-30 mg
Iron	200-400 I.U
Phosphorus	0.30-0.7 mg
Thiamine	0.03-0.4 mg
Carotene	0.046 mg
Riboflavin	40 I.U

Moreover, nutrient content of guava fruit varies across the cultivars of guava. Although, one variety such as strawberry guava contains vitamin C of 90 mg per 100 gm fruit which has found about 25% amount in more known varieties. The amount provides 100% of daily intake for an adult person (Healthaliciousness, 2008). It contains carotenoids and flavonoids. It also contain polyphenol which is the major part of antioxidant pigments that can be possessed of having high potential antioxidant value into the plant foods (Jimenez-Escrig et al., 2001). Fruit skin and color are produced by these pigments. For that guavas color is formed. Guava which are red orange in color that contain pigment such as carotenoid, pro-vitamin A and polyphenol, retinoid than the fruits with yellow green colored (Joseph and Priya, 2011).

2.4 Physico-chemical properties of guava fruit

Physical parameters of guava fruit and chemical parameters also are included in the following table:

Table 2.2: Physico-chemical properties of *Psidium guajava* (Chavan et al., 2016)

Physical properties (fruit)	**Content**
1. Color	Greenish yellow
2. Average weight	138 g
3. Diameter	4.6 cm
4. Recovery to pulp	93.5
5. Other (skin, seeds)	6.4
Chemical properties (pulp)	**Content**
1. Moisture	85.61 %
2. TSS	12°B
3. Acidity	.81 %
4. Reducing sugar	5.1 %
5. Total sugar	7.1 %
6. Ascorbic acid	243 (mg/100g)

2.5 Medicinal properties of Guava fruit

The long history of guava's utilization has driven latest specialists to contemplate guava extracts. Its customary use for looseness of the bowels, gastroenteritis and other stomach related objections has been approved in various clinical investigations. Clinical study related to infantile diarrhea with the fruit juice has been occurred. In a clinical examination with 62 newborn children with childish rotavirus enteritis, the recuperation rate was 3 days (87.1%) in those treated with guava, and loose bowels stopped in a shorter timespan than controls. It was deduced in the examination that guava has great healing impact on juvenile rotavirus enteritis. In two randomized human investigations, the utilization of guava fruit for 12 weeks was appeared to diminish blood pressure by average 8 points, decline overall cholesterol levels by 9%, decline triglycerides by nearly 8%, and increment HDL cholesterol by 8%. The impacts were ascribed to the high potassium and fiber content of the guava fruit (1-2 pounds of guava was eaten every day by the study subjects to get these outcomes). It has been documented that the guava fruit or its juice can lower blood glucose levels in diabetic and normal animals and humans. Guava (*Psidium guajava*) has antioxidant, anti-diabetic, antibacterial, anti-diarrheal, anti hypotensive, analgesic and anta

provocative, anticancer, anti-hypertensive, antifungal, antipyretic and high dietary benefit. The entire fruit of this plant is palatable. The fruit guava can be eaten cooked or raw. The fruits can be used as salads or may be desserts by cutting into slices. Refreshments are likewise arranged from the pulp of this whole fruit for making beverages (Conde, 2003).

The main constituents of guava are vitamins, tanins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. These and other compounds are related to many health effects of guava (Haida et al., 2011).

Some authors have found high concentrations of carotenoids (beta-carotene, lycopene, and beta-cryptoxanthin), vitamin C and polyphenols in guava (Oliveira et al., 2010). Lycopene has been correlated with the prevention of cardiovascular damage because of its positive effects on dyslipidemia. Ascorbic acid is recognized for its important antioxidant effects.

Isolation of nine triterpenoids from guava fruit were done and ursolic acid and other triterpenoids are associated with anti-cancer properties (Shu et al., 2006).

Thuaytong and Anprung (2011) found antioxidant activity in guava and the major constituents identified in white and red guavas were ascorbic acid, gallic acid, catechin equivalents, cinnamyl alcohol, ethyl benzoate, ß-caryophyllene, (E)-3-hexenyl acetate and α-bisabolene. The antioxidant properties of the guava pulp can be related to anticancer effects (Bontempo et al., 2012).

Animals treated with guava pulp juice had significantly lower body weight, glycemia, cholesterol and triglycerides levels and significantly augmented the levels of HDL cholesterol when compared to the animals from the control group (Farinazzi et al., 2012). Lyophilized pulp of guava in diabetic rats induces to significant hypoglycemic effects probably due to its antioxidant activity of compounds present in the pulp (Huang et al., 2019).

2.6 Indian olive fruits in brief

Indian olive is a minor fruit in Bangladesh. Minor fruits are very much appreciated throughout the world for the nutritional profile of them and their medicinal value. Those fruits are considered as a significant dietary source of fiber, bioactive compounds and phyto-chemicals. For their nutritional value, minor fruits are

considered as a nutritious food and those are effective against degenerative diseases which are associated with aging those are occurred by toxic radicals. Comparing minor fruits with other fruits, these contain high antioxidant activity and abundant in various phytochemicals especially phenolic compounds (Francesca et al., 2012).

Indian olive (*Enaeocarpus floribundus*) pertains to the *Elaeocarpaceae* is popular as name of jalapi in Bangladesh and northern regions of West Bengal. These fruits are used for the preparation of chutney, pickles and various culinary purposes. Flowers of the Indian olive appear during summer seasons (April to May). The jalpai fruits are harvested on August to October. These are greenish fruit with single seed, drupe. The mesocarp which is the edible portion of the fruit remains around the seeds (Bhowmick, 2017).

2.7 Physico-chemical characteristics of Indian olive

Some physical parameters of Indian olive fruits like fruit weight, length and bread was determined. Bio-chemical parameters were recorded in the following table;

Table 2.3: Physico-chemical characteristics of *Enaeocarpus floribundus* (Bhowmick, 2017).

Parameters	Values
Fruit weight	22.4 g
Fruit length	4.49 cm
Fruit breadth	2.89 cm
No of fruits	2609
Yield (kg/tree)	58.60
TSS	10.73° B
Total sugar	6.92 %
Ascorbic acid	14.97 mg/100g
Reducing sugar	2.32

2.8 Benefits of Indian olive

Bioactive compounds are rich in olive fruit extract and the fruit extract possesses antibacterial activity in opposition to food-borne bacteria. The plant can be important for the production of antibacterial agents which are non-antibiotic. And also useful in

the storage of food. The phenolic compounds related scientific investigation of olive fruits, oil of olive and leaves of olive plant are available in literatures which done for the antioxidant activities (Sircar and Mandal, 2017).

In earlier, the antimicrobial activity in vitro has been reported for the olive leaves by the researchers (Zaman, 2016). The olive fruits in aqueous form possessed the ability to make stable nanoparticles of silver which had antibacterial effect in opposition to pathogens that are drug resistant (Kahil et al., 2014).

There have been a report related for the olive fruits and leaves of *Olea europaea L* that they contain compounds which can act against infections of microbial (Kubo et al., 1985).

From Sircar and Mandal (2017), a study was performed to screen the bioactive components which can be present in the Indian olive fruit extract that were locally available. In that study the evaluation of growth inhibition against pathogenic bacteria which are food borne also was done. That was done to identify the agents those are the great source for antibacterial components to be conducted in opposition to such infection. In most of the incidents, food poisoning take places every year for the reason of bacterial pathogens namely *Bacillus cereus,* Salmonella spp*, Staphylococcus aureus,* Campylobacter spp*, Clostridium botulinum, Escerechia coli, Clostridium perfringes, Vibrio parahaemolyticus* Which are normally identified in raw foods. So, scientific studies about new antimicrobials occurred. (De Boer et al., 2001)

Medina et al. (2007) stated the efficacy of virgin olive fruit extracts in aqueous in opposition to pathogens related to food borne illness such as *Salmonella enteritidis* and *Listeria monocytogenes*. So, scientific research has to done for this purpose to avoid and prevent the alarming cause of food-borne illness to combat the situation. It also reported that the olive fruit oil has the potentiality of inhibiting the growth of some foodborne bacteria and the olive leaf extracts various bioactive components like flavonoids, tannins, terpenoids, phenol, steroids, glycosides, etc.

Various phytochemicals present in olive fruit extract which are active in opposition to bacterial pathogens and makes them capable in antibacterial activity. Specified phenolic components were quantified and recognized from olive leaves extract which possessed antimicrobial properties. The bacteriological efficacy of different table olives natural black olives and black ripe olives having seven phenolic components

that have been exhibited against gram positive and gram negative bacteria (Pereira et al., 2006).

In the extract of olive fruit in both aqueous and ethanol had a mass range of bioactive components and detected it in quantitative analysis. So the fruits might be significant in the preparation of antibacterial agents those are non-antibiotic to be administered in opposition to bacterial infection and in case of storing of food as well (Sircar and Mandal, 2017).

2.9 Fruit toffee

Toffee is a significant sugar confectionary product. It is accounted for that pulpy fruits like mango, guava, papaya, fig, jackfruit and so forth can be used for processing of toffee. Fruits are sapid and can be used in fresh, value added categories and processed. These are monetarily significant and healthfully key nourishment ware. Man has kept these products in his eating routine to give assortment, taste, intrigue, stylish intrigue and to meet certain healthful prerequisites. Toffees made from fruits normally are extremely nutritious as they have a large portion of the constituents of natural product from which they are made (Kohinkar et al., 2014). Toffees are the chewable sugar confectionary containing sugar, milk solids and margarine or vegetable fat as the significant fixings. Because of their taste and flavor, they appreciate wide prominence. This prominence could be put to appropriate use by expanding the nutritive estimation of toffees as far as proteins, minerals and nutrients. In mixed fruit toffee, the mixing of fruit contributes towards improving the protein and mineral substance of the toffees. Furthermore an assortment of flavors can be gotten with fresh fruit pulp in toffee (Sucheta et al., 2018).

Fruit toffee is a dried mash with legitimate measure of sugar and mixture of acid. Mixing of fruits will bring about great blend of taste and nutritive worth. These things can discover great attractive incentive in future. The confectionary items are exceptionally mainstream among the kids all through the world because of their taste and flavor. Toffee is one of the sugar based items which is to a great extent devoured. The regular toffees are commonly produced using sugar, skim milk powder, spread and other colors made synthetically and synthetic flavor may be also used. In the present study, the endeavors have been executed to incorporate the characteristic of guava and olive mash in the toffee and to assess its quality (Domale et al., 2008)

2.10 Mixed fruit toffee processing and preparation

In one study, preparation of mixed fruit toffee from guava and strawberry was performed. They assessed the changes in chemical components and tangible properties of toffee during storing at surrounding just as refrigerated conditions. It was discovered that TSS and all out sugars expanded with the progression of capacity period while moisture content, ascorbic acid and acidity diminished. The sensory nature of toffees likewise diminished at quicker rate during stockpiling period at encompassing condition than the refrigerated condition (Chavan et al., 2016).

In another study, arrangement of blended natural product toffee from fig and guava was obtained. They evaluated the adjustments in chemical components of fig and guava fruit toffee and found that moisture of toffee diminished negligibly from 8.8 to 7.7%, T.S.S (from 83.4 to 86.4º Brix) and all out sugars incremented because of decline in moisture content; titrable acidity diminished quickly due to the hydrolysis of non-reducing sugars (Kohinkar et al., 2014).

From one of the investigation, preparation of toffee from fig and mango and the changes in nature of prepared toffees during storage was done (Sakhale et al., 2012). Essentially processed custard apple toffees by mixing custard apple mash and different ingredients in the proper proportions (Dhamul et al., 1996).

Guava toffees were processed by blending pulp with sugar, fluid glucose, and milk powder and Vanaspati ghee to the mixture (Sivakumar et al., 2007). Innovation for improvement of delicate date toffee was institutionalized (Khapre and Shah, 2016). They found that toffees had great tangible qualities with moisture of 7.2 percent, amount of ash 2.2 percent, protein 4.5 percent, fat 8 percent, TSS 86° Brix, pH 5.9. Delicate date toffees were also made (Shah et al., 2011). Delicate dates were utilized as a wholesome advancement of sugar and protein. Arrangement of toffee from guava and determination of the best blend of 500 g sugar, 100 g fat, 100 g SMP per kg of the pulp was performed in another investigation (Chavan et al., 2016).

Peach-soy mixed toffees were made by mixing peach and soybean slurry in the proportions of 100:0, 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30 followed by storage for four strategies (Anisa et al., 2016). Mixed toffee from mango and papaya was processed in various extents alongside 16 treatment mix with four degrees of mango

and papaya proportion and four degrees of sugar (Kumar et al., 2017). It was accounted for that treatment mix of 60% mango pulp + 40% papaya pulp with 400g sugar was generally satisfactory if there should arise an occurrence of organoleptic parameter. Though, mix of 80%mango + 20% papaya pulp with 400g sugar was discovered best regarding flavor and 20% mango + 80% papaya pulp with 200g sugar was best in appearance. Physical compound examination and sensory assessment of the peach-soy toffees were reported that there was an expansion in TSS, corrosiveness, sugars and reduction in protein, fat and sensory parameters in various mixes of toffees during four months of capacity.

Tamarind, mango and papaya mash was utilized to get ready mixed fruits toffee (Nale et al., 2007). Pulps were mixed in various proportions like, 100:0 (just tamarind), 50:50 (blend of tamarind and mango) and 75:25 (mix of tamarind and papaya). It was discovered that mix containing tamarind and mango in the proportion of 50:50 was generally satisfactory as far as item quality and tactile characteristics. Attri et al. (2014) arranged and assessed storage stability of papaya toffee for a half year and found that there was increment in the degrees of moisture and diminishing sugars though decline in all out sugars, carotenoid substance and calcium content was taken note. Roy and Singh (1979) arranged bael toffees by blending bael mash with sugar, glucose, skim milk powder and hydrogenated fat. A slight adjustment was done in the arrangement that is expansion of sulfur dioxide to the item. It was accounted for that sulfur dioxide improved nature of the toffee as well as forestalled non-enzymatic searing during a half year stockpiling period.

2.11 Effect of storage on physico – chemical composition of toffee

Preparation and estimation of storage stability of papaya toffee for six months and found that there was increase in the levels of moisture content and reducing sugars whereas decrease in total sugars, ascorbic acid, carotenoid content and calcium content was noticed (Attri et al., 2014).

Physico- chemical analysis and sensory evaluation of the peach- soy fruit toffees (Anisa et al., 2016) revealed that there was an increase in TSS, acidity, sugars and decrease in protein, fat, ascorbic acid and sensory properties in different blends of toffees during four months of storage.

Physico- chemical analysis of mixed fruit toffee (mango and papaya pulp) was prepared and revealed that TSS of mixed fruit toffee increased with the increase in mango pulp, sugar content and storage period. Per cent titrable acidity increased whereas pH and ascorbic acid decreased with the increase in storage period (Kumar et al., 2017). Determination of the changes in chemical composition of fig and guava mixed fruit toffee was performed and found that moisture content of toffee decreased minimally from 8.8 per cent to 7.7 per cent, T.S.S (from 83.4 to 86.4º Brix) and total sugars increased due to decrease in moisture content; titrable acidity decreased rapidly and reducing sugars increased 10 due to the hydrolysis of non-reducing sugars during storage (Kohinkar et al.,2014). Similar trend in the chemical properties of mixed toffee made from guava and strawberry was observed (Chavan et al., 2015).

A decrease in total sugar content was reported in guava toffees. Chemical attributes of papaya and apricot mixed fruit toffee showed different trend during storage. T.S.S, acidity, total sugars, carotenoids and vitamin-C levels decreased during storage whereas reducing sugars increased during 6 months of storage. No changes were observed in ash and fiber content of toffees during storage (Sivakumar et al., 2007).

2.12 Effect of storage on organoletic qualities of toffee

In one study, there was decrease in quality parameters of guava toffee with increase in storage period was reported (Sivakumar et al., 2007). Color, taste and texture were significantly affected during storage period. From an investigation, highest overall acceptability score of 8.30 of mixed fruit toffee prepared by peach pulp and soy slurry (85:15 ratios) was recorded (Anand et al., 2009). Toffee prepared from fig and guava in 75:25 ratio was most acceptable under ambient and refrigerated conditions at the end of six months storage (Kohinkar et al., 2014). Similar trend in overall acceptability was noticed in mixed toffee from guava and strawberry (Chavan et al., 2015).

Different results were reported for papaya toffee (Attri et al., 2014). Colour, flavour, texture, taste, and overall acceptability decreased significantly during three months of storage and these values were further decreased at the end of the six month of the storage; except colour which was found to be negligible.

Chapter-3: Materials and Methods

3.1 Location of the study area

The experiments were conducted in the laboratories of the department of Applied Food Science and Nutrition, Food Processing and Engineering, Biochemistry lab and Poultry research and Training Centre lab, Quality Control and Analytical Lab at Chattogram Veterinary and Animal Sciences University (CVASU).

3.2 Collection of raw materials

Guava and olive fruits were collected from the local market of Chattogram city. Other ingredients like brown sugar, skimmed milk powder, butter, salt, glucose were brought from the renowned supershop namely khulshi mart near the study area.

3.3 Methodology

The study design and sample size which were selected for organizing the study was:

3.3.1 Study Design

In this study, randomized control trial was used for conducting the experiments. The formulation was taken randomly for the ratio of guava and Indian olive pulp to prepare and process the mixed fruit toffee. The creation of four formulation was done by adding 100% guava pulp in the toffee which was indicated as control and other three formulations were created with 80% guava with 20% Indian olive , 70% guava with 30% Indian olive and 40% guava with 40% Indian olive. The ratio was selected randomly and comparison among four formulations was performed.

3.3.2 Sample size

For the purpose of analyzing data from desirable parameters, four formulations were tested triplets. All the data analysis were done by evaluating the triplet values of each sample for determining the mean value.

3.4 Preparation of mixed fruit toffee

For preparing mixed fruit toffee at first all of the raw materials were collected then materials were processed. All of the processed raw materials were blended and heated till the desirable TSS (total soluble solids) level of the mixture. Then it was cooled and left for about 4 hours to be set. After producing final product this was packed with suitable packaging materials and lastly stored at normal temperature.

3.4.1 Materials and Ingredients

Ingredients needed for the preparation of the toffee are enlisted:

- Guava
- Indian olive
- Butter
- Brown sugar
- Salt
- Glucose
- Skimmed milk powder (SMP)

Materials needed for the preparation of the toffee are enlisted:

- Chopper
- Chopping board
- Blender
- Weight machine
- Spatula
- Cooking pan
- Induction Heater
- Refractometer
- Baking paper
- Aluminium foil
- Tray
- Knife

3.4.2 Extraction of fruits pulp

Guava and Indian olive fruits with firm texture, uniform in size were used for the experiment. The fruits were washed under tap water. After drying of the fruits, they were cut into pieces with using chopper in a chopping board and were blended in an electric blender to obtain fine pulp.

3.4.3 Formulation of mixed fruit toffee

Table 3.1: Formulation of mixed fruit toffee

Ingredients	Samples			
	S_1(control)	S_2	S_3	S_4
Guava Pulp (%)	100	80	70	60
Indian Olive pulp (%)	00	20	30	40
Brown sugar (%)	60	60	60	60
Butter (%)	10	10	10	10
Glucose (%)	10	10	10	10
SMP (%)	16	16	16	16

3.4.4 Process Flow diagram for preparing mixed fruit toffee

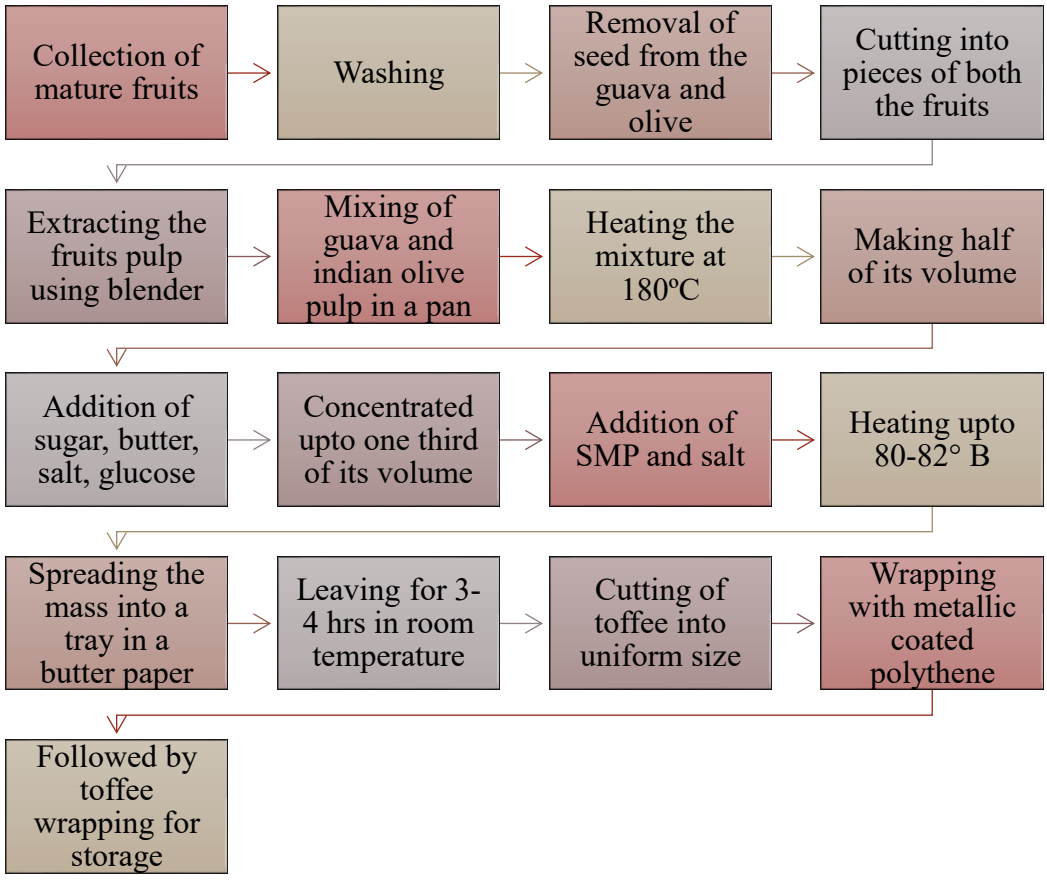


Figure 3.1: Process Flow Diagram for Preparing Mixed Fruit Toffee

Description of Process steps for Preparation of Mixed Fruit Toffee:

1) Selecting and washing

Fully matured green colored guava and matured Indian olive were selected for the preparation of mixed fruit toffee from the nearest shop. The fruits were free from any damage and defect. So they were healthy fruits and ready for further stage of preparation. The fruits were washed with potable water to remove dust, sand and any debris attached to it.

2) Cutting and removal of seeds

Both the fruits were cutting into pieces with the help of a knife and keeping them into a chopping board. After that removal of seeds were done.

3) Preparation of pulp

Guava pulp was extracted using electric blender. Olive pulp was obtained also using the same processor before that olive pieces were steamed with water.

4) Weighing of the ingredients and pulp

After extraction of both the pulp, they were weighed in the electric balance. Then, other ingredients which were beneficial for the preparation of toffee were also weighed.

5) Heating

The pulps were mixed at a ratio of maintaining the formulation. The mixture then heated at 180°C in an induction heater. They were heated till half of their volume were reduced.

6) Addition of other ingredients

When the mixture reached to about half of its volume, ingredients like glucose, brown sugar, butter were added to the mixture of the pulp. The mixture was continuously stirred with spatula. Concentrating upto one third of its volume, addition of SMP and salt was occurred.

7) Setting of toffee

The mass was heated to a thick consistency (80-82 ºB). After that, spreading the thick mass as a sheet in a stainless steel tray over a baking paper or aluminum foil at 2 cm thickness. Let the mass to be dried and set in a cool place for about 3-4 hrs.

8) Cutting of toffee

After setting of the toffee, it was cut into uniform size and ready for packaging.

9) Packaging

Butter paper and metallic coated polythene wrappers, aluminum foil and polythene gauge bags were obtained from the market.

10) Storing

Lastly, the wrapped toffee was stored at room temperature.

3.5 Proximate analysis

Proximate analysis of developed mixed fruit toffee was conducted by determining the parameters including moisture content, crude fiber, crude protein, fat, carbohydrate by following the AOAC method of certain edition which was indicated in the procedures of the analysis.

3.5.1 Moisture content

The moisture was measured by oven drying at 105°C to constant weight (AOAC, 2016).From the start, weight of void pots were dried and 5gm of test was put on it. At that point the pot was put in an air stove (thermostatically controlled) and dried at temperature of 105°C for 24 hrs. In the wake of drying, the pot was expelled from the stove and cooled in desiccator. It was then weighed with spread glass. The cauldron was again set in the stove, dried for 30 minutes, removed from the dryer, cooled in desiccator and gauged. Drying, cooling and weighing were rehashed until the two back to back loads were same.

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

3.5.2 Protein

Reagents used: Concentrated H_2SO_4, Digestion mixture (Potassium sulphate 100gm + Copper sulphate 10gm + Selenium dioxide 2.5gm), Boric acid solution, Alkali solution, Mixed indicator solution, Standard HCl (0.1N)

For estimation of protein, the steps were followed:

Digestion: 2g sample, 3g digestion mixture and 25 ml H_2SO_4 were taken in a kjeldahl digestion flask. It was heated for 4 hours in a kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 100ml water, 100 ml 40% NaOH and glass blitz were added to kjeldahl flask which containing about 10 ml 2% boric acid and 2-3 drops mixed indicator. About 100 ml distillate was collected just before the distillation was stopped. The receiving flask was moved so that the tip of the distilling tube was out the distillate. Some distillate was collected in this way to make sure the condenser tube was free from traces of ammonia.

Titration: The ammonia collected was titrated with 0.1N HCl solution and titer value was recorded. The calculation of the percent of protein in the sample using protein factor 6.25.

3.5.3 Fat

All out lipid was extricated by the AOAC (2016) technique utilizing the Soxhlet mechanical assembly. The dried sample staying after dampness assurance was moved to a thimble what's more, stopped the highest point of the thimble with a wad of fat free cotton. The thimble was dropped into the fat extraction tube joined to a Soxhlet flask. Around 75ml or a greater amount of anhydrous ether was filled a flask. The highest point of the fat extraction tube was connected to the condenser. The sample was separated for 16 hrs or longer on a water bath at 800°C. Toward the finish of the extraction time frame, the thimble was expelled from the mechanical assembly and refined off a large portion of the ether by permitting it or gathered in Soxhlet tube. The ether was poured off when the tube was almost full. At the point when the ether arrived at a little volume, it was filled a little, dry measuring utencil through a little pipe containing an attachment of cotton. The flask was flushed and separated

altogether, utilizing ether. The ether was dissipated on a steam bath at low heat; it was then dried at 100ºC for 1hr, cooled what's more, weighed. The distinction in the loads gave the ether dissolvable material present.

The presence of fat was expressed as follows:

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

3.5.4 Ash

The ash content of the samples was determined by the standard AOAC method (AOAC, 2003). This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash. Briefly, five grams (5g) of sample was burned and put into muffle furnace with crucible at 550ºC for 8 hrs It was calculated using the following formula:

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

3.5.5 Crude fiber determination

Crude fiber was determined according to AOAC method (2005). Crude fiber is the water insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose and lignin. It is estimated through digestion of fat free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained. Following apparatus are used: Liebig condenser, Reflux condenser, Gooch crucible.

Reagent required:

1. 0.255N Sulphuric acid solution
2. 10.0% Potassium sulphate solution;
3. Asbestos- Gooch grade.

Calculation: The loss in weight represents crude fiber

$$\% \text{ crude fiber } = \frac{\text{weight of residue with crucible} - \text{wt of ash with crucible} \times 100}{\text{Wt. of sample (moisture and fat free)}}$$

3.5.6 Determination of total carbohydrates

It was given as the difference between 100 and a sum total of the other proximate components. Hence it was calculated using the formula below (Southgate, 1969):

% CHO= 100- (Protein+ Fat+ Fiber + Ash + Moisture content) %

3.6 Estimation of Vitamin C

Vitamin C content had been estimated by using dye method (King, 1941). Dye solution was to be taken to the burette upto 0 mark. Then 5 ml vitamin c solution was taken in the conical flask. The conical flask had to be placed under the burette and the dye was added drop wise. Titration was completed when pink color was appeared and stayed for 20 seconds and then disappeared. The reading should be taken for at least 3 times. The result should be expressed as mg/100g.

Reagents required:

1. Dye solution
   - 2, 6- dichlorophenol indophenols (260 mg)
   - $NaHCO_3$(210mg)
2. Metaphosphoric acid solution
   - Metaphosphoric acid (15 mg)
   - Glacial acetic acid (40mg)
3. Standard ascorbic acid solution

3.7 Mineral content analysis

Mineral contents were determined by using biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay. For sample preparation, 5 g of sample was taken into a conical flask. After that, 7.5 ml HNO_3 and 2.5 ml $HCLO_4$ was added into the conical flask. Then it as heated over an induction cooker at 200W until complete digestion. Then it was cooled. Finally, deionized water was added upto 100ml. The results were expressed as mg/100g after conversion from mg/dl.

3.8 TSS determination

Hand refractometer was used for determination of TSS. It is based on the principle of total refraction. Few drops of distilled water was placed on the prism. The distilled water reading should be zero then chamber was cleaned with muslin cloth. A drop of sample was placed on the prism.

3.9 Antioxidant capacity evaluation

DPPH Assay: Antioxidant capacity of the extracts was determined using DPPH assay as described by (Azlim Almey et al., 2010) with slight modifications. Stock solution (1 mg/mL) of extract was diluted to concentrations of (0.50, 1.00, 1.50, 2.00, 2.50) mg/mL in methanol. Methanolic DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL methanol. The methanolic DPPH solution (2 mL) was added to 1 mL of each extract solution of different concentrations and the mixture was left for 30 min and the absorbance was read at wavelength 517 nm. Control was prepared by mixing 1 mL of methanol with 2 mL of DPPH solution. Methanol was used as a blank while Trolox was used as a standard. Antioxidant capacity based on the DPPH free radical scavenging ability of extracts was calculated and expressed as milligrams of Trolox equivalents (TE) per gram of extracts (mg TE/g).

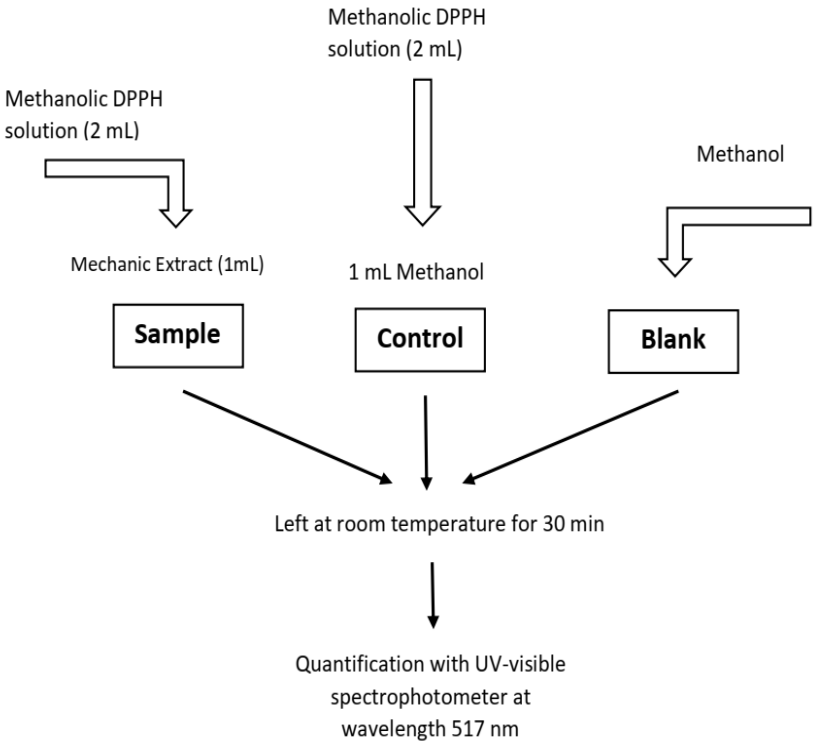


Figure 3.2: Determination of antioxidant capacity

3.10 Microbial analysis

1. TVC test
2. E.coli detection
3. Fungal Test

Microbial Analysis of the samples was done in the Poultry Research and Training Centre (PRTC), Chattogram Veterinary and Animal Sciences University.

3.10.1 Determination of total viable count

Total viable count also known as (TVC) gives a quantitative idea about the presence of microorganisms such as bacteria, yeast, and mold in a sample. To specific, the count actually represents the number of colony forming units (cfu) per gram (or per ml) of the sample. A TVC is achieved by plating dilutions of the culture until 30-300 colonies exist on a single plate. A high TVC count is usually attributable to poor quality. Procedure was followed from Modern Food Microbiology Jay, J.M. (1995).

Procedure:

At first a series of test tubes each containing of 9 ml diluents were taken. 50 gram/ml food sample was homogenized in 450 ml diluents and making suspension in a beaker. From the original sample, 1ml was transferred in the test tube no. 1 and mixed thoroughly. Transferred 1 ml from 1st test tube to 2nd test tube and continue up to last one and 1ml discarded from the last test tube. From each test tube 3 petri dishes were taken containing PCA media. Then transfer 0.5 ml mixture from each of the test tube to the corresponding petri dish separately. Diluted samples should be spread over the surface of the media using glass spreader. The petri dishes were kept in incubator in inverted position at 37°C for 2/3 days. After 1 day interval up to 3 days after incubation the colonies were observed. In which plate colony counted are 30-300 should be included and others should be discarded. The three petri dish colony of each tube is counted and made average to them.

3.10.2 *E. coli* detection

E. coli, test portion, initial suspension, and sufficient number of dilutions were made following the standard method. Double-and single-strength Lauryl sulfate tryptosebroth, EC broth, and Brilliant green lactose bile broth were made as confirmation mediain McCartney bottle or screw cap tube with inverted Durham tube.

Three tubes of double-and single-strength liquid selective enrichment medium were then inoculated with a specified quantity of the test sample or with a specified quantity of an initial suspension and incubated at 30°C or 37°C for 24 hr. or 48 hr. A series of tubes of the confirmation medium were inoculated with the cultures from the tubes of double-and single-strength selective enrichment medium in which gas formation or opacity preventing the detection of gas formation has been noted. The most probable number of coliforms per milliliter or per gram of sample (i.e., the MPN) was calculated from the number of tubes in the new series showing gas formation. A table for determination of most probable numbers was used (Feng et al., 2002).

3.10.3 Fungal test

Fungal test was done for prepared product. Procedure was followed from Modern Food Microbiology Jay, J.M. (1995)

For fungal test Sabouraud Dextrose Agar and following procedure was used: **Media/ Agar:** Sabouraud Dextrose Agar

Agar preparation:

65gm agar was dissolved in 1litre distilled water. Then boiled it completely it was sterilized by autoclaving at 121 °C for 15 minutes. Then poured it onto the petri dish.

Test procedure:

Just few sample was set up in center of the petri dish then it was incubate at 25 °C for 5 to 7 days after incubation the result was observed.

3.11 Energy estimation

The energy content of the chocolate carrot bar 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).

Energy = (Protein × 4.1) + (Fat × 9.3) + (Carbohydrate × 4.1)

3.12 Cost analysis

Cost of the toffee made from guava and olive were calculated from the overall ingredients cost which were utilized for the preparation of the mixed fruit toffee. The amount was presented in taka and analyzed for the price of per piece of toffee.

3.13 Sensory evaluation

Sensory evaluation was performed for the determination of overall acceptability of the final product by the consumers. A taste-testing panel evaluated the consumer's acceptability of developed product. The panel test was done in the CVASU premises where the panelists were both the teachers and students of CVASU. Panelists of 30 persons were given the product that has been developed from the fruits of guava and Indian olive. There were four formulations which were encoded with sample-1, sample-2, sample-3 and sample-4. The four samples were tasted by the panelists without informing them the formulations. The panelists were requested to assign appropriate score for sensory attributes of appearance, color, flavor, texture, taste, sweetness and overall acceptability of mixed fruit toffee. This method does not, of course, reflect actual consumer perception, but it does strongly indicate attributes which a good quality product should possess (Sing *et* al., 2008). They tasted four toffees expressed their opinion giving score about. The scale were arranged such that:

Table 3.2: Rating Scale for sensory evaluation

Ranks	**Scores**
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slight	4
Dislike moderately	3
Dislike very much	2
Dislike Extremely	1

3.14 Statistical analysis

Analytical data (proximate composition, mineral contents, antioxidant capacity, vitamin C estimation, sensory evaluation) were determined and stored in Microsoft excel spread sheet to evaluate statistical analysis. All samples were in three replicates. Descriptive statistics (mean, standard deviation) were performed to for the data

analysis of mixed fruit toffee. Data was sorted, coded and recorded in IBM SPSS statistics 16. After that statistical analysis was conducted. Proximate composition, mineral content, vitamin c, sensory evaluation, antioxidant capacity data were analyzed by using Oneway ANOVA procedures to assess significant level of variation at 95% confidence interval. Post hoc “Tukey” was conducted to identify the variation within the sample groups. The statistical analysis was obtained at 5% level of significance ($p<0.05$).

Chapter-4: Results

4.1 Proximate analysis

The proximate composition of for four type of formulation of mixed fruit toffee prepared from four different mixtures of guava and Indian olive pulp was shown in table 4.1. Sample 1 contained lowest mount of moisture content (16.88±.36) % whereas sample 3 contained the highest amount of it (23.52±.47) %. The carbohydrate amount in mixed fruit toffee samples were determined in extensive amount. In sample-1, carbohydrate content (67.43±1.2) % was in highest position. On the other hand, sample-4 contained lesser amount of it (57.13±.47) %. Ash content was highest (1.68±.22) % in sample 2 and lowest (1.41±.18) in sample 3. A one way analysis of variance (ANOVA) was carried out for the four samples data analysis. Descriptive statistics and Post hoc Tukey test in this procedure was conducted to analyze the data at 5% level of significance.

Table 4.1: Proximate analysis of mixed fruit toffee

Sample id	Moisture (%)	Carbohydrate (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Fiber (%)
S_1	16.88±.036^{c}	67.43±1.2^{a}	4.9±0.45	4.88±.43^{a}	1.67±.31	4.24±0.14^{d}
S_2	21.64±1.14^{b}	59.73±1.3^{bc}	5.2±0.36	3.59±0.17^{c}	1.68±.022	8.16±0.30^{b}
S_3	23.52±0.47^{a}	59.92±0.90^{b}	4.6±0.26	4.13±0.15^{bc}	1.41±0.18	6.42±0.19^{c}
S_4	21.88±0.25^{ab}	57.13±0.47^{c}	5.3±0.31	4.71±0.11^{ab}	1.67±0.15	9.26±.018^{a}
P-value	0.000	0.000	0.123	0.001	0.425	0.000
Significance	***	***	NS	**	NS	***

*** Significant at P<0.001 ** Significant at P <0.01; * Significant at P <0.05; NS= no significance at P>.05. Values followed by different superscript letters denote a significant difference; comparison done across formulation

Legends: S_1= (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava

pulp+40% Indian olive). All values are showed in ME±SD of data where ME= Mean and SD= Standard Deviation.

4.2 Mineral content analysis

Fruits are basically rich in mineral contents. But as this product is mixed with other ingredients the mineral content was not significant as the fresh fruit contents. Fro, table 4.2, sample 1 contained highest (213.33±15.27) mg/100g amount of calcium content in mg per 100 gm of the product and lowest in sample 2 (156.67±20.82) mg/100g. On the other hand, magnesium content was much (323.33±58.59) mg/100g in sample 4 and least (166.67±15.27) mg/100g at sample 2. Phosphorous content was in least amount in sample 1 and much in sample 4. However, potassium (106.67±32.14) mg/100g and iron content (66.66±15.27) mg/100g seemed to be higher in sample 3 than any other samples though they were not significant and these constituents were lowest in sample 1.

Table 4.2: Mineral contents of mixed fruit toffee

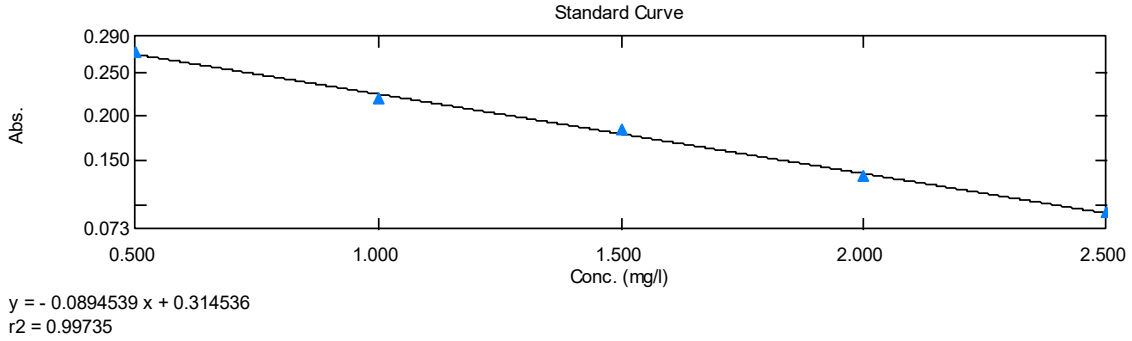
Mineral (mg/100g)	**Amount**				**P-value**
	S_1	S_2	S_3	S_4	
Calcium	213.33±1.27^{a}	156.67±20.82^{b}	170±2.00^{ab}	186.67±2.82^{ab}	0.034
Magnesium	193.33±2.82^{bc}	166.67±15.27^{c}	283.33±0.35^{ab}	323.33±5.59^{a}	0.002
Phosphorous	76.67±5.77^{b}	93.33±5.77^{ab}	106.67±2.14^{ab}	133.33±1.55^{a}	0.023
Potassium	60±1.0	63±5.77	66.66±1.27	66.65±1.77	0.821
Iron	11±0.50	17±0.033	19±0.4	13±0.77	0.678

*** Significant at P<0.001 ** Significant at P <0.01; * Significant at P <0.05; NS= no significance at P>0.05. Values followed by different superscript letters denote a significant difference; comparison done across formulation.

Legends: S_1= (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive). All values are showed in ME±SD of data where ME= Mean and SD= Standard Deviation

4.3 Antioxidant capacity of the final product

From the table 4.3, it was observed that antioxidant capacity was significantly highest (30.9±.014) TE/100g in sample-3 and significantly lowest in sample-4 (21.6±.092) TE/100g in correspondence to the absorbance which were 0.038±.002 and 0.121±.009 TE/100g respectively.



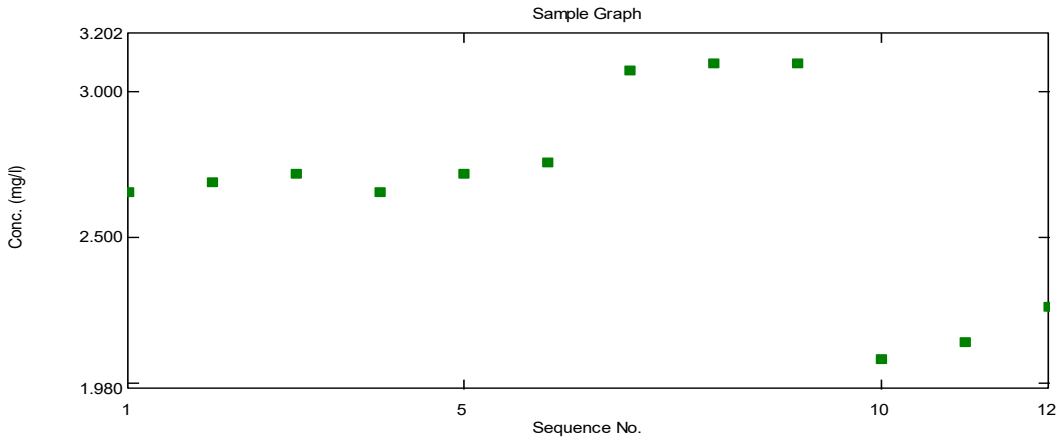


Figure 4.1: Graphical representation of antioxidant capacity determination

The graphical representation was observed to determine the antioxidant value where standard curve for trolox and curve for sample against the standard shown in which x-axis was presented for absorbance and y-axis was presented for corresponding concentration.

Table 4.3: Antioxidant capacity of mixed fruit toffee

Parameters		**Antioxidant capacity (mg TE/100 g)**	**Wavelength**
Sample code	S_1	26.8±.032[b]	0.074±.003[b]
	S_2	27.1±0.051[b]	0.072±.004[b]
	S_3	30.9±.0014[a]	0.038±.002[c]
	S_4	21.6±0.092[c]	0.121±.009[a]
P-value		0.000	0.000
Significance		***	***

*** Significant at P<0.001 ** Significant at P <0.01; * Significant at P <0.05; NS= no significance at P>0.05. Values followed by different superscript letters denote a significant difference; comparison done across formulation

Legends: S_1 = (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive). All values are showed in ME±SD of data where ME= Mean and SD= Standard Deviation.

4.4 TSS of final product

From the table 4.4, it was reported that TSS (total soluble solids) was highest (82 degree brix) in sample 2 and lowest in (80 degree brix) in sample 3.

Table 4.4: TSS of developed mixed fruit toffee

Sample Id	TSS (°B)
S_1	81
S_2	82
S_3	80
S_4	81

In the table, S_1 = (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive).

4.5: Vitamin C content in the developed product

Guava and Indian olive fruits are abundant in vitamin C content. Guava contains vitamin C in most of its composition. From the table 4.5, in the mixed fruit toffee the vitamin C content was significantly highest 91.66±1.66 mg/100gm in sample-4 and lowest (50±2) mg/100gm in sample-1 which was found significant.

Table 4.5: Vitamin C content in mixed fruit toffee

Parameters		Vitamin C (mg/100g)
Sample id	S_1	50 ± 2^{b}
	S_2	75 ± 5^{a}
	S_3	66 ± 2^{a}
	S_4	91.66 ± 1.66^{ab}
P-value		0.000
Significance		***

*** Significant at P<.001 ** Significant at P <0.01; * Significant at P <0.05; NS= no significance at P>.05. Values followed by different superscript letters denote a significant difference; comparison done across formulation

Legends: S_1= (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava

pulp+40% Indian olive). All values are showed in ME±SD of data where ME= Mean and SD= Standard Deviation.

4.6 Microbiological analysis of the product

Table 4.5 revealed that, sample 1, sample 2, sample 3, sample 4 did not contain any *E.coli* and *Salmonella* spp. Other evaluation on total viable count and fungal count also determined from o to 30 days after preparation of the toffee. The sample were stored in the ambient temperature for 30 days for the evaluation. The presence of yeast and mold were not exist when the products were produced and after 1 month their presence had not been identified.

Table 4.6: Microbiological evaluation of mixed fruit toffee

Microbiological properties	**Duration**	**Sample**			
		S_1	**S_2**	**S_3**	**S_4**
E.coli		ND	ND	ND	ND
Salmonella		ND	ND	ND	ND
TVC (CFU/gm)	0	1×10^3	1.6×10^2	1.4×10^3	1.2×10^2
	After 1 month	3.5×10^3	6.8×10^2	5.5×10^3	4.8×10^2
Fungal (yeast and mold)	0	Negative	Negative	Negative	Negative
	After 1 month	Negative	Negative	Negative	Negative

In the table, S = (100% guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20%Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive).

4.7 Cost analysis

Table 4.7: Cost analysis of four mixed fruit toffee samples

Sample ID	Heads of expenditure		Amount (gm)	BDT	Total cost (TK)
S_1	1.Ingredients	Guava pulp	1000	100	425
		Indian Olive pulp	000	000	
		Brown sugar	600	45	
		Butter	100	90	
		Glucose	100	40	
		SMP	160	140	
	2.Processing cost 15% of raw materials cost				64
					489
S_2	1.Ingredients	Guava pulp	800	80	430
		Indian Olive pulp	200	25	
		Other ingredients same as S_1	Amount as S_1	325	
	2. Same as S_1				65
					495
S_3	1.Ingredients	Guava pulp	700	70	430
		Indian Olive pulp	300	35	
		Other ingredients same as S_1	Amount as S_1	325	
	2. Same as S_1				65
					495
S_4	1. Ingredients	Guava pulp	600	60	435
		Indian Olive pulp	400	50	
		Other ingredients as S_1	Amount as S_1	325	
	2. Same as S_1				66
					501

In the table, S_1= (100% guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20%Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive).

By following this recipe with the similar amount of pulp 1 kg, 100 toffees can be prepared. So, price of per piece toffee is:

For sample-1, per piece toffee = 489/100 tk

= 4.89 tk

For sample -2, per piece toffee = 495/100 tk

=4.95 tk

For sample -3, per piece toffee = 495/100 tk

=4.95 tk

For sample -4, per piece toffee = 501/100 tk

=5.01 tk

4.8 Energy content determination

From the figure 4.3, Energy content in sample 1 was calculated in highest amount (341.94 kcal/100g) and lowest (299.6 kcal/100g) in sample 2.

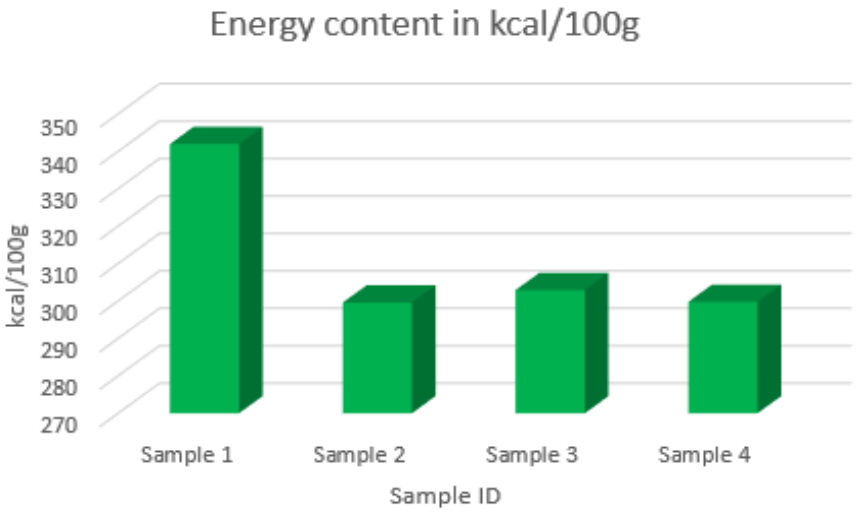


Figure 4.3: Comparison of energy content among four mixed fruit toffee

4.9 Sensory evaluation of mixed fruit toffee

From table 4.7, highest scores for color were recorded (8.2±0.78) in case of sample 1 and lowest (6.73±1.39) scores was recorded for sample 3. Not only for color but also for all the sensory attributes such as appearance, flavor, texture, taste and overall acceptability sample 1 got highest position among the four samples of mixed fruit toffee prepared from the guava and jalapi pulp. On the other side, sample 3 got the lowest position and lowest scores at every aspects of the sensory attributes.

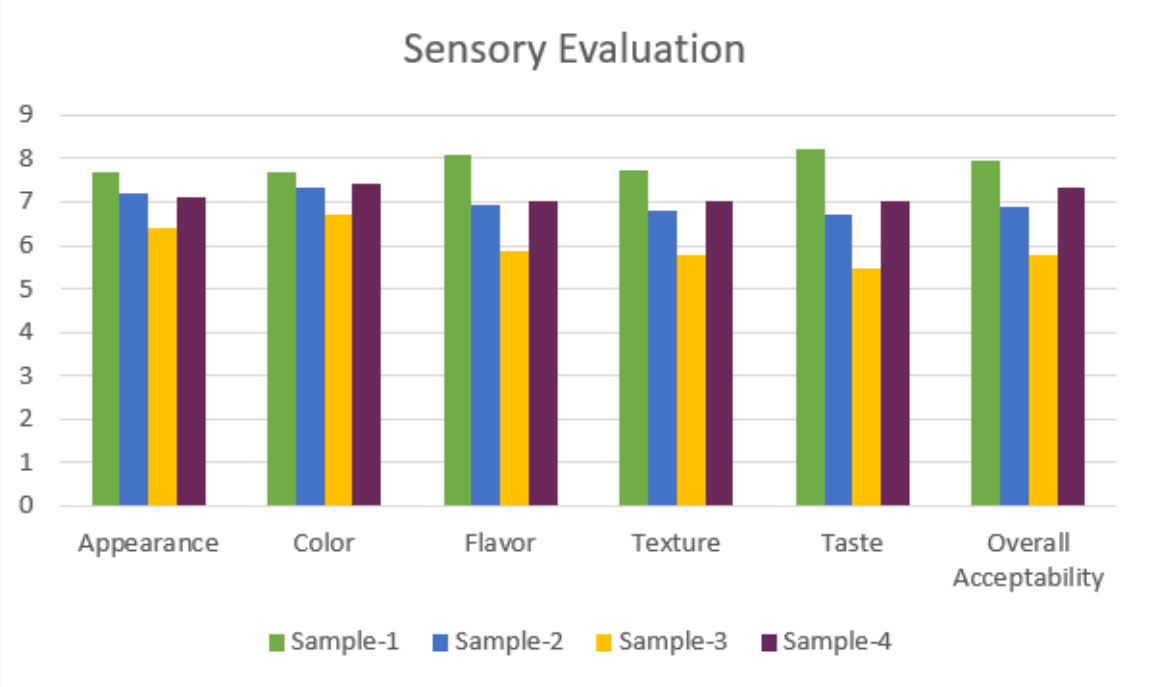


Figure 4.4: Comparison on sensory attributes among four toffee samples

Table 4.8: Hedonic rating test for sensory evaluation of mixed fruit toffee

Sensory Attributes	Sample				P-value
	S_1	S_2	S_3	S_4	
Appearance	7.67 ± 0.82^{a}	7.2 ± 1.01^{ab}	6.4 ± 14^{b}	7.13 ± 0.91^{ab}	0.020
Color	7.67±1.11	7.33±1.4	6.73±1.39	7.4±0.98	0.217
Flavor	8.07 ± 0.70^{a}	6.93 ± 1.38^{bc}	5.87 ± 1.25^{c}	7.00 ± 1.00^{ab}	0.000
Texture	7.73 ± 0.96^{a}	6.80 ± 1.32^{ab}	5.80 ± 1.57^{b}	7.00 ± 1.00^{a}	0.001
Taste	8.20 ± 0.68^{a}	6.73 ± 1.5^{b}	5.47 ± 1.36^{c}	7.00 ± 1.25^{b}	0.000
Overall Acceptability	7.93 ± 0.79^{a}	6.87 ± 1.41^{ab}	5.8 ± 1.47^{b}	7.33 ± 0.98^{a}	0.000

*** Significant at P<.001 ** Significant at P <0.01; * Significant at P <0.05; NS= no significance at P>0.05. Values followed by different superscript letters denote a significant difference; comparison done across formulation

Legends: S_1= (100%guava pulp+00% Indian olive=control), S_2= (80%guava pulp+20% Indian olive), S_3 = (70%guava pulp+30% Indian olive), S_4 = (60%guava pulp+40% Indian olive). All values are showed in ME±SD of data where ME= Mean and SD= Standard Deviation

Chapter-5: Discussions

5.1 Proximate analysis

Combination of guava pulp and Indian olive pulp in development of toffee provides the basic nutrients like protein, fat, fiber and some minerals. The proximate composition of four types of formulation of mixed fruit toffee prepared from four different mixtures of guava and Indian olive pulp was shown in table 4.1.Sample 1 contained lowest amount of moisture content (16.88±0.36) %. In this study, moisture content ranged from 16.88±0.36 to 23.52±0.47 %. Khapre (2010) studied that the chemical characteristics of guava-soy toffee was including moisture 14.8% which was similar to the lowest value of moisture content in this study. Anisa et al. (2016) revealed the proximate composition of peach-soy toffee included moisture content 14.9% this also showed similarities with the present study. The moisture content of the developed toffee was slightly higher than the reports of other studies. Moisture content is an important factor in maintaining food quality because increase moisture facilitates the growth of microbes and ultimately destroy quality.

The carbohydrate amount in mixed fruit toffee samples were determined in extensive amount. In sample-1, carbohydrate content (67.43±1.2) % was in highest position and sample-4 contained lesser amount of it (57.13±0.47) %. The carbohydrate amount determined at the range of 57% to 67.43%. From the study of Khapre (2010), carbohydrate in the guava-soy toffee was found 62.3% which was similar with the present study. The carbohydrate may be come from the addition of sugar and glucose into the toffee during processing.

Protein content was highest in sample-4 (5.3±0.31) % and lowest in sample-3 (4.6±.26) %. Shakale et al. (2012) stated that the toffee prepared from blend of 80:20 (fig : mango) resulted into high protein content (6.3%), which could be due to higher protein content in fig than in mango and this report was little bit higher than the current investigation. On the other hand, one of an investigation showed that hard toffee prepared from pineapple and palmyrah contained protein 4.4% whereas soft toffee contained protein 1.77% (Thanusan et al., 2018). The amount of protein in soft toffee from pineapple and palmyrah was analogous with the present investigation. In case of hard toffee, the amount of protein was much lower from the present study.

Ash content was highest in sample-4 (1.68±0.22) % and lowest in sample-3 (1.41±0.18) %. The ash was determined at the range of 1.41 to 1.68% from the table 4.1.). Khapre (2010) studied that in the guava-soy toffee ash was about 3.3% which was much higher than the present study. From another study of Thanusan et al (2018), the ash was determined 1.43% for toffee prepared from pineapple and palmyrah. It showed similarities with current study.

Fiber content in sample-4 was the highest (9.26±.18) % content among four samples and the lowest (4.24±.14) % amount belonged to the sample-1. The fiber content was in the range of 4.24 % to 9.26% from the table 4.1. On the other hand, one of an investigation showed that hard toffee prepared from pineapple and palmyrah fiber 1.53%, ash 1.43% whereas hard toffee contained fiber 1.94% (Thanusan et al., 2018). By comparing with this study, fiber content was much higher in the present developed toffee.

Furthermore, fat content was highest in sample-1(4.88±0.43) % and lowest (3.59±0.17) % in sample-2. The fat was evaluated in the range of 3.59% to 4.88%. Khapre (2010) studied that the fat content of guava-soy toffee was 11.1% which showed dissimilarities with the present study. Another study revealed the proximate composition of peach-soy toffee included 8.46% fat content (Anisa et al., 2016) and this was much higher than the current study. As fruit and vegetable contain less protein and crude fat, these constituents were found not such a significant amount in the four samples.

5.2 Mineral content in mixed fruit toffee

The ash content is inorganic residue remaining after the organic matter has been burnt away. It is not necessarily of exactly the same composition as the mineral matter present in the fresh pulp as there may be losses due to volatilization or some interactions between constituents. Mineral content of formulated toffee types was measured for potassium, magnesium, phosphorus, iron and calcium and the values were reported in table 4.2. In that table, it had been denoted that calcium content was 213.33 mg/100 g for sample-1 which was the greater value and 156.57 was the lesser value present in sample-2. In sample-4, phosphorus content was found 133.33 mg/100g, was the highest amount. Sample-4 also possessed highest amount of potassium (66.65 mg/100g) and magnesium (323.33mg/100 g). A report was shown as Potassium 29 mg/100g, sodium 21 mg/100g, phosphorous 180 mg/100g, iron 34

mg/100g in terms of hard toffee and Potassium 29 mg/100g, sodium 28 mg/100g, phosphorous 100 mg/100g, iron 18 mg/100g in case of soft toffee processed from pineapple and palmyrah (Thanusan et al., 2018). By comparing the mineral content with the developed toffee, it can be said that the amount was dissimilar and it may be due to the variation in fruit pulp nutrients used for the preparation of the toffee.

5.3 Vitamin C content

From the table 4.5, it was seen that vitamin c content was higher in sample-4 (91.66±1.66 mg/100g) and lower in sample-1 (50±2 mg/100g). The ascorbic acid content ranged from 50 to 91.66 mg/100gm among the four samples. Ascorbic acid content was 13.8 (mg/100g) in guava-soy toffee (Khapre, 2010) which was lower than the amount found in present study. Chavan et al. (2016) evaluated ascorbic acid 89.35 mg/100g in guava toffee which was quite similar to this study. In one study of processing of papaya toffee, ascorbic content was estimated 21.6 mg/100mg and much lower than the present study because papaya contains lower amount of vitamin c. (Atrri et al., 2014). Anisa et al. (2016) reported that the highest ascorbic-acid content of 21.80 mg/100 g was found in peach pulp and soy-slurry toffees of 70:30 ratio and the lowest 15.04 mg/100 g was found in control. Kaushal and Batt (1999) reported increase in ascorbic acid content of sprouted soy-slurry fortified fruit bar. So it can be stated that the amount of ascorbic acid in the developed product was quite good and greater than the former studies.

5.4 Antioxidant capacity of mixed fruit toffee

The antioxidant activities were showed differences among the toffee types. DPPH is an extensively used substrate to evaluate antioxidant activity especially for investigating the free radical scavenging activities of biological as well as chemical substances. Antioxidant capacity content was higher (30.9±.014) mg/ml in sample 3 and lower in sample 4 (21.6±.092) mg/ml. Thanusan et al. (2018) reported in their study that the DPPH scavenging activity of soft toffee and hard toffee were 10.48±.34 mg/ml, 10.28±.07 mg/ml which were prepared from palmyrah and pineapple mixture. By comparing the two investigations, antioxidant capacity was superior in the developed mixed fruit toffee from guava and Indian olive than toffee processed from palmyrah and pineapple.

5.5 Microbial analysis of mixed fruit toffee

Microbial counts were determined by using standard plate count (SPC). This study was shown that there was no growth of bacteria, yeast, and mold during thirty days of storage where the product was kept in vacuum packaging at room temperature (30 ± 2°C). It can be said that the final products are safe for human consumption and free from microbial contamination.

5.6 Sensory evaluation of the toffee

Sensory analysis of toffee blends was done to obtain the most organoleptically acceptable proportion amongst all the toffee blends. Sensory analysis data from table 4.7 shows that toffee blend containing 100 per cent guava pulp (sample-1) that means which was control and scored highest for all the parameters. It had an overall acceptability of 7.93±0.79 which was significantly higher than all other formulations except sample- 4. The overall acceptability level of sample-4 was nearest with the value of sample-1. The sample-1 got the highest scores at all perspectives such as appearance, color, flavor, texture, taste and overall acceptability of sensory attributes. However, the result showed that there was no undesirable score for any mixed fruit toffee that indicates all the fruit toffee samples are acceptable corresponds to their sensory characteristics.

The mixture containing 70 per cent guava pulp and 30 per cent Indian olive pulp showed least scores for all parameters with 5.8±1.47 as the overall acceptability score which is still in the acceptable range. Kohinkar et al. (2014) found that the toffee prepared by using 75: 25 % blend (fig: Guava) scored highest (8.6), while toffee having 25: 75 % blend (fig: guava) scored minimum score (8.0). From another study, results of sensory evaluation palmyrah: pineapple at 60:40 mixed fruit toffee was selected as best composition because it was scored the highest average ranking score for their all the sensory attributes (Thanusan et al., 2018). Anisa et al. (2016) revealed in their study that in case of overall acceptability, the highest score of 8.30 was recorded in 85:15 ratio and the lowest score of 6.01 in 70:30 ratio in peach-soy toffees respectively. The reason could be assigned to the fact that toffees prepared from 85:15

ratio of peach pulp and soy-slurry had a better consistency and flavor due to an ideal ratio of blend. From the investigation of Khapre (2010), highest total sensory score obtained by T3 treatment showed that 15% soya slurry with 85% guava pulp was the best combination for the preparation of guava-soya toffees. By comparing with other studies, it has been clear that fruit pulp which was used as base in the mixed toffee got highest acceptability than other formulations. So, similarity in sensory evaluation with other investigations has been found.

Chapter-6: Conclusion

The findings of the present study indicated that the mixed fruit toffee sample-1(100% guava) got the superior position in case of overall acceptability though sample-4 (60% guava + 40% Indian olive) was close enough with it. In nutritional perspective, sample-4 was possessed the highest position. This can be due to the addition of both the fruits in an appropriate proportion that balanced the nutrients properly. The cost of sample-1 was less than any other formulations. The price of the other toffee may be little bit high due to the addition of Indian olive and there was no flour or volume increaser was used to increase the volume of the toffee. The produced mixed fruit toffee was prepared without using any synthetic chemicals and preservatives. As it is a sugar confectionary item, it contains a large amount of sugar where sugar acts as a natural preservative. No artificial flavor and color were added in the mixed fruit toffee that can define it as a natural processed product and it also produced a natural flavor of fruit with desirable color which was significantly acceptable to the panelists. Sample-4 contains highest amount of potassium, magnesium and phosphorus. Ash and fiber content was determined highest amount in sample-4 which can be due to the addition of both nutritious fruits. Sample-4 was also rich in vitamin C. Vitamin C content may highest in sample-4 because of containing both guava and Indian olive fruit pulp in the toffee which contain a great amount of vitamin C. Microbial growth in developed mixed fruit toffee was not in deteriorating condition and within the acceptable limit.. Thus, it can be concluded that mixed fruit toffee of sample-4 can be the best choice as it got good scores in sensory evaluation that was quite similar to the highest score of sample-1 and also possesses highest nutritional quality containing high amount of protein, ash, fiber, magnesium, phosphorous, potassium and vitamin C. Moreover, it can be stored for 1 month at ambient temperature without losing its quality and can be consumed for the betterment of human health.

Chapter-7: Recommendations and future perspectives

At present people are obsessed with processed foods including toffees and chocolates made with cocoa. In this age, natural fruit toffee prepared from fruit pulp can be a good option for them. By using fruits instead of cocoa that is processed worldwide can give people more nutritious processed food item. Further study can be proceed by adding food preservative to increase the shelf life of the product and also the product can be stored at refrigerator for furthermore investigations.

The product texture was not so good as it was prepared manually during heating. So there is in need of using advanced food processors to manufacture better quality toffee and to feel better on the mouth and increment of its texture.

In addition, other formulations can be done by using more olive in the toffee to get more sour taste as the present study was performed on the base of guava but olive can be used as the base with the guava fruits.

However, some physicochemical parameters can be determined in the future study to get a tremendous job in this developed product. By increasing shelf life, texture and nutritional quality of the developed product, the enriched product can be manufactured commercially and may be it can gain popularity among the consumers.

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Appendix A: Photo Gallery

Fresh fruits

Cutting of fruits

Pulping

Weighing

Cooking

Spreading

Cutting

Packaging

Figure : Preparation of mixed fruit toffee

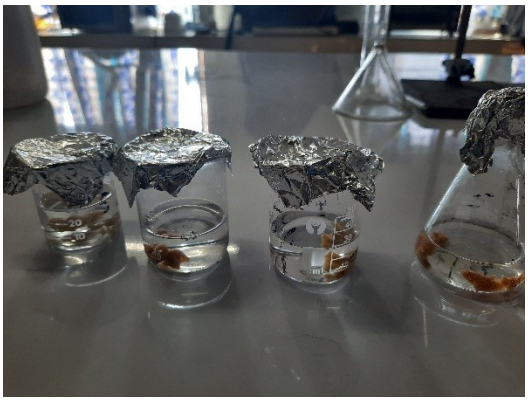
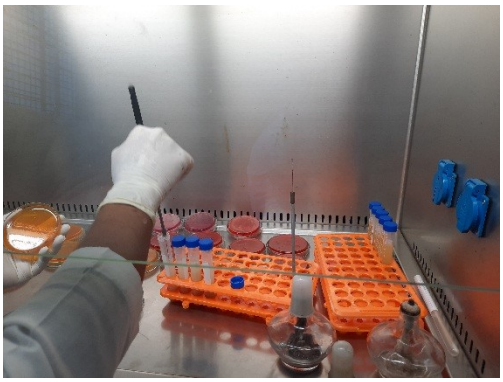
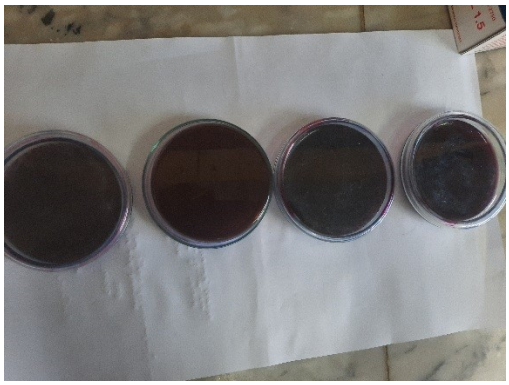


Figure : Determination of antioxidant capacity





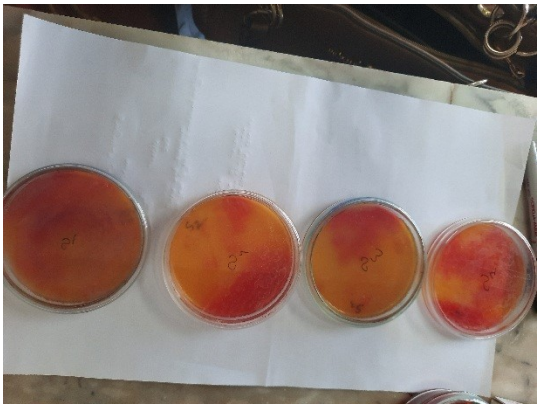


Figure : Microbial activity analysis

Figure : Determination of Mineral contents

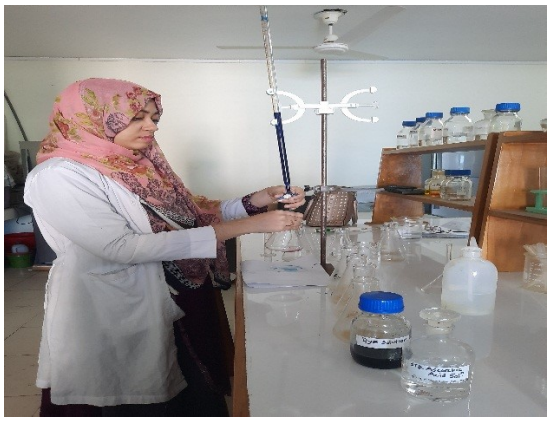


Figure : Estimation of Vitamin C content

Figure : Paneltest for the final product

Appendix B: Calculation of energy content in mixed fruit toffee

For sample-1, Energy = (Protein × 4.1) + (Fat × 9.3) + (Carbohydrate × 4.1)

= (4.9 × 4.1) + (4.88 × 9.3) + (67.43 × 4.1)

= 341.94 kcal/100g

For sample-2, Energy = (Protein × 4.1) + (Fat × 9.3) + (Carbohydrate × 4.1)

= (5.2 × 4.1) + (3.59 × 9.3) + (59.73 × 4.1)

=299.6 kcal/100g

For sample-3, Energy = (Protein × 4.1) + (Fat × 9.3) + (Carbohydrate × 4.1)

= (4.6 × 4.1) + (4.13 × 9.3) + (59.92 × 4.1)

=302.94 kcal/100g

For sample-4, Energy = (Protein × 4.1) + (Fat × 9.3) + (Carbohydrate × 4.1)

= (5.3 × 4.1) + (4.71 × 9.3) + (57.13 × 4.1)

=299.77 kcal/100g

Appendix C: Hedonic Rating Test (Mixed Fruit Toffee)

Name: **Age:** **Sex:** **Date:**

Please Taste these samples and let me know whether you like or dislike these. Show your attitude by checking at the point that best describe your feelings about the samples through using the appropriate scale. Consider sample 1,2,3,4 starting from left to right of your position. Kindly keep your honest review on this product. Put your score followingly:

Hedonic	**Appearance**				**Color**				**Flavor**				**Texture**				**Taste**				**Overall Acceptability**			
	Sample				**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**	**1**	**2**	**3**	**4**
Like extremely																								
Like very much																								
Like moderately																								
Like slightly																								
Neither like nor dislike																								
Dislike slightly																								
Dislike moderately																								
Dislike very much																								
Dislike extremely																								

Comments (if any):

N.B: Overall scale used: 9= like extremely; 8=like very much, 7= like moderately; 6= like slightly; 5= neither like nor dislike; 4= dislike slightly; 3= dislike moderately; 2= dislike very much; 1= dislike extremely

Brief Biography

Miftahul Zannat passed the Secondary School Certificate Examination in 2009 and then Higher Secondary Certificate Examination in 2011. She obtained her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University (CVASU). Her research interests are development of food products, evaluation of nutritional quality of food products, clinical dietetics, human nutrition, new techniques to measure food quality, taste and flavor.