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Competitive Research Grant (CRG)

Sub-Project Completion Report

on

Study on the nutritional quality of underutilized mulberry fruits, leaves & silkworm pupae and their value addition

Project Duration

July 2017 to September 2018

Bangladesh Sericulture Research and Training Institute (BSRTI)

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
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Project Implementation Unit
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
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Acronyms

AOAC : Association of Official Analytical Chemists
BSRTI : Bangladesh Sericulture Research and Training Institute
BSDB : Bangladesh Sericulture Development Board
BSTI : Bangladesh Standard & Testing Institution
BCSIR : Bangladesh Council of Scientific and Industrial Research
BARC : Bangladesh Agriculture Research Council
CP : Crude protein
CF : Crude fiber
CatE : Catechine Equivalents
DG : Director General
DNS : Di-nitro salicylic acid
DPPH : 1-diphenyl-2-picryl-hydrazyl
GAE : Gallic Acid Equivalents
MFP : Mulberry Fruit Pulp
MTS : Mulberry Tea Samples

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Executive Summary

The present work was undertaken to evaluate the nutritional quality of underutilized mulberry fruits, leaves and their value added products. Nutritional facts of silkworm pupae were also evaluated. Mulberry fruit Juice (MFJ) was extracted for mulberry jelly preparation. Out of a total of eight different treatments for standardization of the recipe of mulberry jelly the recipe with MFJ + 50% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate scored highest in organoleptic quality test. For the preparation of sauce from mulberry fruits, four different combinations were tested out of which the recipe containing 80 parts of MFJ and 20 parts of Water (80:20) along with other ingredients (salt, sugar, vinegar, citric acid, and different spices and 1% sodium benzoate) scored highest in organoleptic quality test. Mulberry jelly prepared according to final recipe was found to contain iron and calcium at the rates of 1.21 and 22.63 mg/100 g respectively. Total carbohydrate content of the jelly was 56.43% with the vitamin C content of 2.32 mg/100g. Mulberry sauce was found to contain iron and calcium at the rate of 1.19 and 24.29 mg/100 g respectively. Total carbohydrate content of the sauce was 53.37% and vitamin C content was estimated to be 1.76 mg/100g. Nutritional quality of mulberry leaves showed significant differences among the leaf maturity stages. Highest moisture (77.32%) and crude protein content (18.91%) were obtained in tender leaves and lowest in coarse leaves (69.14%) and (15.40%) respectively. Maximum amount of minerals (11.65%) was found in coarse leaves and minimum in tender leaves (9.81%). The reducing sugar (1.84%), total sugar (3.58%) and starch (13.27) content were observed significantly higher in coarse leaves compared to the other leaf at maturity level. Moreover, it was also found that the moisture and crude protein contents of mulberry leaves decreased with the increase of leaf maturity level whereas the reducing sugar, total sugar and starch contents were increased with the increase of leaf maturity. Mulberry tea was prepared by using three types of mulberry leaves such as; mixture, tender and mid-mature leaves. Tea prepared with the mixture of tender and mid-mature leaves (MTS-1) and tender leaves (MTS-2) performed best when their quality assessed and compared with BSTI standard for black tea. In the study of antioxidant activity, mulberry tea sample MTS-1 showed the highest DPPH free radical scavenging activity (44.47%). Total phenolic content (28.09 mg GAE/g) and flavonoids content (28.01 mg CatE/g) was also found highest in MTS-1. Nutritional fact study of silkworm pupae showed crude protein percentage to be 43.3 and 40.87 for female and male respectively. Oil content was 26.11% and 21.44% for female and male pupae respectively. But after oil extraction crude protein was significantly increased in the residue of the pupae powder which was 60.56% and 56.28% in case of female and male pupae respectively. The highest extractability of oil was observed with petroleum ether (26.11%) in case of female pupae followed by n-Hexane (21.91%) with hot condition. Oil extracted using petroleum ether detected four extra fatty acids (Palmitoleic acid, Margaric acid, Heneicosanoic acid and 9, 12, 15 Octadecatrienoic acid) compared to n-hexane. Silkworm pupae oil was found to contain 44.154% Oleic acid, 20.44% stearic acid, 17.50% palmitic acid and 15.13% linoleic acid in case of extraction with petroleum ether solvents.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project:
Study on the nutritional quality of underutilized mulberry fruits, leaves & silkworm pupae and their value addition

2. Implementing organization:
Bangladesh Sericulture Research and Training Institute (BSRTI)

3. Name and full address with phone, cell and E-mail of PI/Co-PI (s):

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4. Sub-project budget (Tk):
4.1 Total: Tk 4612333.00
4.2 Revised (if any):

5. Duration of the sub-project:
5.1 Start date (based on LoA signed): 12 July, 2017
5.2 End date : 30 September 2018

6. Justification of undertaking the sub-project:

Mulberry is the sole source of food material for silkworm, *Bombyxmori* L. In nature, there are innumerable plants that have one or the other medicinal values, beneficial to mankind. In recent years, mulberry green (herbal) tea in the brand name of "Ranowghana" is fast becoming popular in Japan and Thailand as it is believed to prevent high blood pressure and diabetes. Besides it also reduces muscle cramp and body pain, reduces the risk of cancer, increases natural activity of body organs and reduces body weight. In China, providing mulberry leaf juice is the most common hospitality in the villages for a visitor. Mulberry fruit is well known as esteemed dessert fruit. In Greece, it is fermented to derive intoxicating beverage. Mulberry fruit is having high source of vitamin C which is commonly used for the preparation of special wine and beer in most of the cold countries. Ripe mulberry fruit can be made use in the preparation of tasty jelly or syrup. *Morusnigra Linn* or black mulberry is now in the headlines for its omnipotent medicinal properties which may even cure the AIDS. Silkworm pupae are being used as a valuable source of nutritious food in china

and Japan. It is reported that it is easily digestible and assimilable with the properties of reducing cholesterol, blood sugar level and provides additional energy. This could be used for extraction of free amino acid, which can be utilized in the pharmaceutical, food and fodder industries as cheap source of raw material.

Organic mulberry tea is rich in variety of nutrients and trace elements that are beneficial to human body. It is natural herbal beverage which can be used for hypertension, hyperlipidemia and hypercholesterolemia. Sericulture developed countries are now giving more attention to diversified product of sericulture. Diversification is the most important component of reaching long-range financial goals while minimizing production cost and risk. If we can use the mulberry fruits, leaves and waste pupae to produce byproducts as well as other than sericulture, scope of additional income of farmers will be generated and they will be more interested to practice sericulture. The present study was undertaken to study the nutritional quality of underutilized mulberry fruits, leaves & silkworm pupae and their value addition.

7. Sub-project goal:

Nutritional assessment of some sericultural byproducts and their value addition.

8. Sub-project objective (s):

- To prepare Jelly and Sauce from mulberry fruits.
- To prepare mulberry tea from mulberry leaves.
- To extract pupae oil form silkworm pupae and study on its fatty acid profile.
- To assess nutritional qualities of each of the byproducts.

9. Implementing location (s):

Bangladesh Sericulture Research and Training Institute, Rajshahi.

10. Methodology in brief:

10.1. Preparation of jelly and sauce from mulberry fruits

Preparation of jelly from mulberry fruits

High yielding and high nutritious mulberry varieties were maintained in the selected experimental plot. Fresh and ripen mulberry fruits were collected from the experimental plot. The technique applied for extraction of mulberry fruit juice (MFJ) is shown in the Figure 1. Known amount of fruit juice were taken and water was added according to recipes and heated up to boiling of water for 10 minutes. Extract was obtained by straining the heated material through muslin cloth without pressing or squeezing.

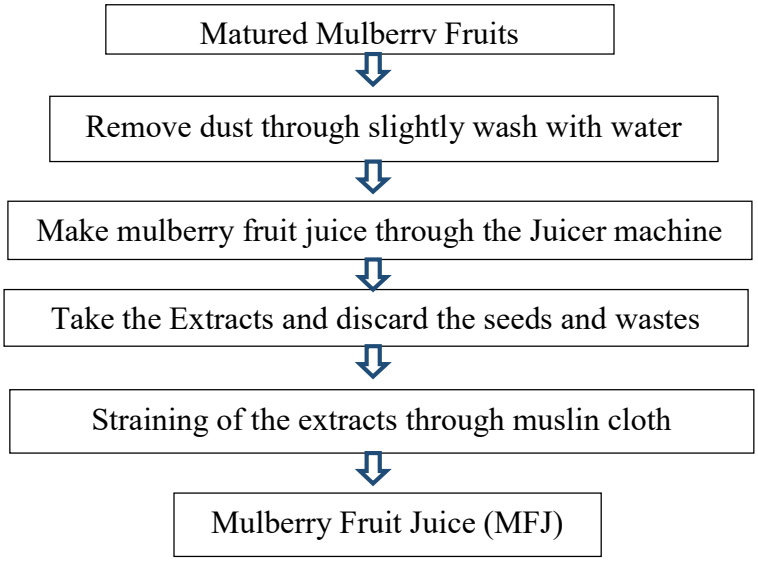

Figure 1: Flow chart for extraction of mulberry fruit juice from mulberry fruits

Method of preparation of mulberry jelly:

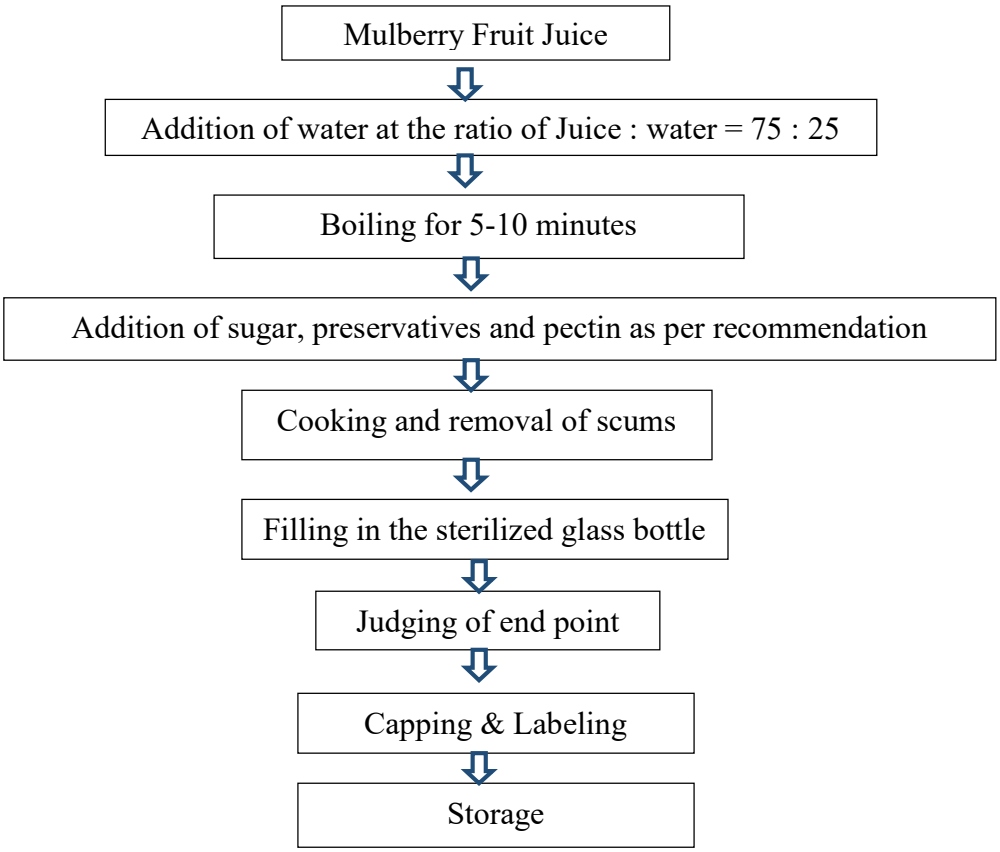

Figure 2: Flow chart for preparation of mulberry jelly

The obtained extract was strained through muslin cloth without squeezing to get the clear extract. Then sugar, pectin, citric acid and sodium benzoate was added with mulberry fruit Juice. The mixture was boiled to the end point to make jelly of desired consistency. Flow chart of the jelly preparation is shown in Figure 2.

For standardization of recipe for mulberry jelly sugar, pectin (food grade), and sodium benzoate (preservative) was added to mulberry fruit Juice (MFJ) in Eight different combinations as follows:

T1: MFJ + 30% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T2: MFJ + 30% sugar + 1.0% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T3: MFJ + 40% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T4: MFJ + 40% sugar + 1.0% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T5: MFJ + 50% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T6: MFJ + 50% sugar + 1.0% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T7: MFJ + 60% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate.
T8: MFJ + 60% sugar + 1.0% pectin + 0.3% citric acid + 0.1% sodium benzoate.

The prepared jelly was filled in sterilized wide mouth jelly bottles (500 g capacity), and sealed air tightly then bottles were covered with lid. The jellies prepared from different recipes were subjected to sensory evaluation on next day for their organoleptic quality such as color & appearance, taste, flavor, texture and overall acceptability by a panel of five persons and the samples were scored on a 9.0 Point Hedonic Rating Scale to find out the best recipe of jelly. Storage life determination was not studied in this experiment due to shortage of time.

Assessment of nutritional content using proximate analysis

To determine the nutritional content of mulberry jelly, the proximate compositions such as; pH, Titratable Acidity (g/100g as citric acid), Total Soluble Solids (TSS)% m/m, ^{0} Brix, Total Carbohydrate%, Total Sugar%, Vitamin-C (mg/100g), Iron (mg/100g), Calcium (mg/100g) were evaluated.

Mulberry Sauce preparation:

This study was conducted in two phases. To prepare sauce from mulberry fruits, the same mulberry fruit Juice (MFJ) was used following the steps in Figure 1 Without strained through muslin cloth.

At the first phase, four sauce formulations were developed by changing the concentration of mulberry fruit pulp (MFJ) as follows:

Recipe-1 (T1): (MFJ:Water 50:50)+ Other ingredients (salt, sugar, vinegar, citric acid, and different spices and 1% sodium benzoate).
Recipe-2 (T2): (MFJ:Water 60:40)+ Other ingredients (salt, sugar, vinegar, citric acid, and different spices and 1% sodium benzoate).
Recipe-3 (T3): (MFJ:Water 70:30)+ Other ingredients (salt, sugar, vinegar, citric acid, and different spices and 1% sodium benzoate).
Recipe-4 (T4): (MFJ:Water 80:20)+ Other ingredients (salt, sugar, vinegar, citric acid, and different spices and 1% sodium benzoate).

At the second phase, sauce formulation was finalized according to their organoleptic quality test score on a 9.0 point Hedonic Rating Scale and physicochemical characteristics were evaluated. Storage life determination was not studied in this experiment due to shortage of time.

To prepare Sauce initially prepared mulberry fruit Juice was boiled with continuous stirring at up to boiling. Then salt, sugar, vinegar, citric acid, and different spices were added as per taste and mixed while boiling. Lastly, 0.1% sodium benzoate was added. The final mixture was boiled with stirring until reaching 25-30 ◦Brix. Then, it was immediately transferred into sterilized clean glass bottles and stored at room temperature.

Proximate analysis

To determine the nutritional content of mulberry Sauce, the proximate compositions such as; pH, Titratable Acidity (g/100g as citric acid), Total Soluble Solids (TSS)% m/m, ^{0} Brix, Total Carbohydrate%, Total Sugar%, Vitamin-C (mg/100g), Iron (mg/100g), Calcium (mg/100g) were evaluated.

Brief procedure of proximate analysis:

pH:

Before taking measurements pH meter was calibrated using standard buffer solution and it was make sure that the samples were at room temperature. pH of the samples were measured with pH meter Hitachi, Horiba, Japan and recorded the value.

Titratable acidity:

Each of 6 grams of sample was taken into 100 ml beaker and 50 ml of distilled water was added into each samples. Samples were then titrated with 0.1 N NaOH to an end point of 8.2 (measured with phenolphthalein indicator) and the volume of milliliters of NaOH was recorded. Then the titratable acidity was calculated using the following formula:

% acid = [mlsNaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100] grams of sample.

Total Soluble Solids (TSS) % m/m, ^{0}Brix:

Degree brix was measured by using Automatic Refractometer, SMART-1, ATAGO, Japan. It was ensured that the juice solution was at a temperature of 20°C. Then one or two drops of sample were placed onto the prism and the prism was closed carefully and reading was taken.

Total Carbohydrate:

Total carbohydrate was measured by Anthrone Method. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides. Carbohydrates were first hydrolysed into simple sugars using dilute hydrochloric acid.

In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green colored product with an absorption maximum at 630nm.
Procedure of carbohydrate measurement:

First Anthrone reagent was prepared by dissolving 200mg of anthrone in 100ml of ice cold 95% H_2SO_4.

Then Standard glucose was prepared by dissolving 100mg of glucose in 100ml water and used as stock solution. From the stock solution of glucose, Working standard of glucose was prepared by diluting 10ml of stock to 100ml with distilled water. Stored refrigerated after adding a few drops of toluene.

Then 100 mg of the sample was taken into a boiling tube and hydrolysed by keeping it in boiling water bath for 3 hours with 5ml of 2.5 N-HCl and cooled to room temperature. Neutralized with solid sodium carbonate until the effervescence ceases. The volume was made up to 100 ml and centrifuged. The supernatant was collected and taken 0.5 and 1ml aliquots for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. '0' served as blank. The volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water and then 4 ml of anthrone reagent was added. Heated for eight minutes in a boiling water bath and cooled rapidly and read the green to dark green color at 630nm. A standard graph was drawn by plotting concentration of the standard on the *X*-axis versus absorbance on the *Y*-axis. From the graph the amount of carbohydrate present in the sample tube was calculated by using the following formula:

Amount of carbohydrate present in 100mg of the sample= (mg of glucose ÷ Volume of test sample) X 100

Total Sugar:

The presence of added sucrose was detected by determining sugars before and after inversion by copper- reduction methods.

Standardization of Fehling's solution: Prepared standard dextrose solution was taken into a 100 ml volumetric flask. Volume of dextrose solution required to reduce all the copper in 10 ml of Fehling solution) was recorded and compare corresponding to the standard dextrose solution from table. Pipette 10 ml of Fehling's solution into a 300 ml of conical flask and run in from the burette. The flask containing mixture was heated over wire gauze. Gently boiled the contents of the flask for 2 minutes. At the end of two minutes of boiling one ml of methylene blue indicator solution was added without interrupting boiling. While the contents of the flask started to boil then standard dextrose solution was started adding (one or two drops at a time) from the burette till blue color of indicator was disappeared. The titration was completed within one minute so that the contents of the flask was boiled together for 3 minutes without interpretation. Total volume in ml of std. dextrose solution used for the reduction of all the copper in 10 ml of Fehling's solution was noted. The reading was then multiply by the number of mg of anhydrous dextrose in one millilitre of standard dextrose solution to obtain the dextrose factor. This factor was compared with the dextrose factor and the correction was determined.

Test sample was transferred representing about 2- 2.5 gm sugar to 200 ml volumetric flask and diluted to about 100 ml and excess of saturated neutral Lead acetate solution was added (about 2 ml is usually enough). Mixed and diluted to volume and then filtered. The first few ml filtrate was discarded. Dry Potassium or Sodium Oxalate was added to precipitate excess lead used in clarification.

25 ml filtrate or aliquot containing (if possible) 50 – 200 mg reducing sugars was taken and titrated with mixed Fehling A and B solution using Lane and Eynon Volumetric method.

(1) Fehling A: 69.28-gm copper sulphate was dissolved ($CuSO_4.5H_2O$) in distilled water. Diluted to 1000 ml. Filtered and stored in amber coloured bottle.

(2) Fehling B: 346 gm Rochelle salt (potassium sodium tartrate) ($K\ Na\ C_4H_4O_6.\ 4H_2O$) and 100 gm NaOH was dissolved in distilled water. Diluted to 1000 ml. Filtered and stored in amber coloured bottle.

For inversion at room temperature, 50 ml aliquot clarified was transferred and deleaded solution to a 100 ml volumetric flask, 10 ml HCl (1+ 1) was added and kept at room temperature for 24 hours. For

immediate inversion, the sample with HCl was heated at 70^0C for 1 hr. Neutralised exactly with conc. NaOH solution using phenolphthalein indicator and diluted to 100 ml. Titrated against mixed Fehling A and B solution.

Reducing and total reducing sugar was calculated as,
Reducing sugar (%) = mg. of invert sugar x vol. made up x 100
TR x Wt. of sample x 1000
Total reducing sugar (%) = mg of invert sugar x final vol. made up x original volume x 100
TR x Wt. of sample x aliquot taken for inversion x1000
Total sugar (as sucrose) (%) = (Total reducing sugar – Reducing sugar) x 0.95 + Reducing sugar.

Vitamin-C:
Starch indicator solution (1%) was prepared. Then 5.00 g potassium iodide (KI) and 0.268 g potassium iodate (KIO_3) was dissolved in 200 ml of distilled water and 30 ml of 3 M sulfuric acid was added. The solution was poured into a 500 ml graduated cylinder and diluted to a final volume of 500 ml with distilled water and mixed well. Then the solution was transferred to a 600 ml beaker and labeled as iodine solution.

0.25 gm vitamin C (ascorbic acid) was taken in a 250 ml volumetric flask and dissolved with 100 ml distilled water. Then diluted to the mark and labeled as vitamin C standard solution and this solution was standardized with iodine solution.

25.00 ml of juice/sauce sample was added to a 125 ml Erlenmeyer flask and titrated with iodine solution until the endpoint was reached with using starch indicator. Then iodine solution was further added to get a color that persists longer than 20 seconds. Final volume of iodine solution was recorded. Same titration was repeated for three times. Amount of vitamin C was calculated using the conversion factor- 1 Mole 0.01 M I_2 equivalent to 1.76 g of Vitamin C.

Fe:
Juice/sauce samples were centrifuged at 15000 rpm for 20 minutes. Supernatant solution was used for analysis. Supernatant solution was pipetted into 100 ml volumetric flask and added 10 ml of hydroxylamine solution and 5 ml of 1, 10- phenanthroline solution. Then 8.0 ml sodium acetate solution was added which produced red colour of ferrous 1, 10 phenanthroline. After addition of the reagents, the samples were kept for 15 minutes before making the absorbance measurement by spectrophotometer (Model: Geneysis 20 spectrophotometer, Thermospectronic, USA) at 510 nm.

Ca:
The determination of calcium in juice/sauce sample was carried out by preliminary separation of calcium as calcium oxalate by the addition of ammonium oxalate and ammonia. This was followed by the addition of perchloric acid which releases the calcium from the inhibiting effect of the oxalate.

A 5ml volume of sample was pipetted into a 10 ml graduated stoppered centrifuge tube and 5 ml of 1% ammonium oxalate and 3 drops ammonia solution was then added. The mixture was shaked well and allowed to stand for 30 minutes and then centrifuged at 300 rpm for 2 minutes. Decanted the supernatant and allowed the tube to drain inverted for 30 seconds. A 0.5ml volume of 4M perchloricacid was added and shaked followed by heating for 1 minute in a boiling water bath. Cooled and diluted to the 10 ml mark with distilled water. The flame photometer was calibrated using a 100mg/l calcium standard solution containing 50 ml 4M Perchloric acid. The sample was aspirated directly into the flame

photometer. The calcium concentration was then calculated by multiplying the Flame Photometer reading on the calibrated scale by the dilution factor.

10.2. Preparation of mulberry tea from mulberry leaves:

Grading, nutritional analysis and selection of mulberry leaves

A selected mulberry plot with high yielding mulberry variety BM-11 was maintained properly throughout the whole year. Bio chemical analysis of different types of mulberry leaves (e.g. tender, mid mature, coarse and mixture) with different age was done for nutritional facts. Different maturity stage of mulberry plant was identified as the number of leaf position of a branch of mulberry plant. Here leaf number 1-6 was consider as tender leaf and similarly 7-11 as mid mature, 17-20 as coarse and 1-20 was considered as mixture leaves. BSRTI recommended pruning schedule was maintained properly and mulberry leaf maturity stages as determined from the date of pruning as follows: Tender leaf- 35 days, mid mature leaf-60 days, and coarse leaf-80 days. As nutritional quality of mulberry leaf, moisture%, mineral%, crude fiber%, crude protein%, reducing sugar%, total sugar%, and starch% were determined. Moisture% of mulberry leaves was determined by conventional procedure (oven dry basis). Mineral% content of mulberry leaves was determined by the method of AOAC (1980). Crude Protein (CP)% of mulberry leaves were determined following the micro-Kjeldhal method (AOAC, 1990) by Kjeltec^{TM} 8200, FOSS analytical A/S and conversion factor used for calculating of crude protein% was 6.25. The crude fiber% (CF) was determined by the method of AOAC through FiberCap^{TM} FT 221, FOSS analytical A/S and was expressed as g/100 (DW). Reducing sugar% and Total sugar% of mulberry leaf was estimated by Di-nitro salicylic acid (DNS) method (Miller, 1972) following the procedure of Loomis and shull (1937). The starch content of mulberry leaf was estimated following the method of Morse (1974).

Three types of mulberry leaves viz; mixture, mid mature and tender were selected for preparing mulberry tea from mulberry leaves and graded as Mulberry Tea sample MTS- 1, MTS- 2 and MTS- 3 respectively. Before plucking mulberry leaves for tea preparation, plot was sprayed by water for preliminary cleaning. After plucking all mulberry leaves were washed individually and then processed for preparation of mulberry tea.

For quality test of mulberry tea samples (MTS), Water Extract (% m/m), Total Ash (% m/m), Water soluble ash of total ash (% m/m), Alkalinity of water soluble ash (as KOH% m/m), Acid Insoluble ash (% m/m), and Crude Fiber (% m/m) were determined through the standards of Bangladesh Standards and Testing Institution (BSTI) for black tea (BDS 1107: 1984). Reference test methods for above parameters have been mentioned in the respected table.

DPPH free radical inhibition%, Total phenolic and total flavonoids % were also analyzed and compared with seven familiar commercial tea brands in BCSIR laboratory, Rajshahi.

DPPH free radical scavenging activity

The antioxidant activity of the extract was determined by the 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Briefly, 100 µL, 200 µL and 400 µL of each tea extracts (100–400 µg/ml) were mixed with 3.8 ml DPPH solution and incubated in the dark at room temperature for 1 h and considered as treatments T-1, T- 2 and T- 3 respectively. The absorbance of the mixture was then measured at 517 nm. Ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from the following formula:

DPPH scavenging effect = $\frac{Control\ OD - Sample\ OD}{Control\ OD}$ x 100

Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. Briefly, 200 µL of different tea extracts (1 mg/ml) were made up to 3 ml with distilled water, mixed thoroughly with 0.5 ml of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 ml of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of Gallic acid equivalent per g dry weight.

Total flavonoid content

The total flavonoid content of crude extract was deter-mined by the aluminium chloride colorimetric method. In brief, 50 µl of tea extracts (1 mg/ml ethanol) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% NaNO2 solution; 0.3 ml of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg catechine equivalent per g dry weight.

10.3. Extraction of oil from silkworm pupae and its fatty acid profile analysis:

Selection of pupae

Pupa is the life stage of silkworm (*Bombyxmori* L) undergoing transformation between immature and mature stage. Silkworm has four life stages: egg, larva, pupa and moth. Among them it is a complete metamorphosis stage. A high yielding silkworm race namely Urboshi was considered for this study. To get pupae this silkworm race was reared with maintaining of proper silkworm rearing management technique.

Pupae were sorted by male and female. Extractability of pupae oil was checked with two different solvents e.g. Petroleum ether and n-Hexane. Fatty acid profile of extracted pupae oil was analyzed.

Extraction of pupae oil

The healthy pupae samples were dried in an oven at 70ºC until constant weight was obtained and moisture content was determined. The dried pupae were finely powdered and stored in desiccator. Pupae oil was extracted from pupae powder with two different solvents viz. petroleum ether (60-80ºC), and n-Hexane under both hot and cold conditions. For hot extraction 5 gm of pupae powder were taken in a extraction thimble and oil was extracted by using soxthlet apparatus. The extraction was continued for 8-10 hours until the solvent was found clear in the extractor. The flask was transferred to a heated water bath and solvent was evaporated through suction pump. Finally the flask was dried in an oven at 70ºC until constant weight was obtained and oil content was then calculated. In case of cold condition; 5 gm of pupae powder was soaked with 100 ml of solvent, shaken with Galenkamp Shaker machine and kept overnight in a dark place. Next day it was filtered using fat free filter paper and the residue was washed with 50 ml solvent and dried in oven at 70ºC until constant weight was obtained.

Statistical Analysis:
For all treatments three replications were considered. Statistical analysis for Mean, Standard Deviation, Standard Error of Mean, CV%, LSD, analysis of variance with Completely Randomized Design was done using statistical analysis software Satistix10, Version 10.0.

11. Results and discussion:

11.1. Preparation of jelly and sauce from mulberry fruits:

Jelly from mulberry fruits:

Standardization of recipe
The sensory scores for different treatments are presented graphically in Figure 3 on 9 point hedonic scale.

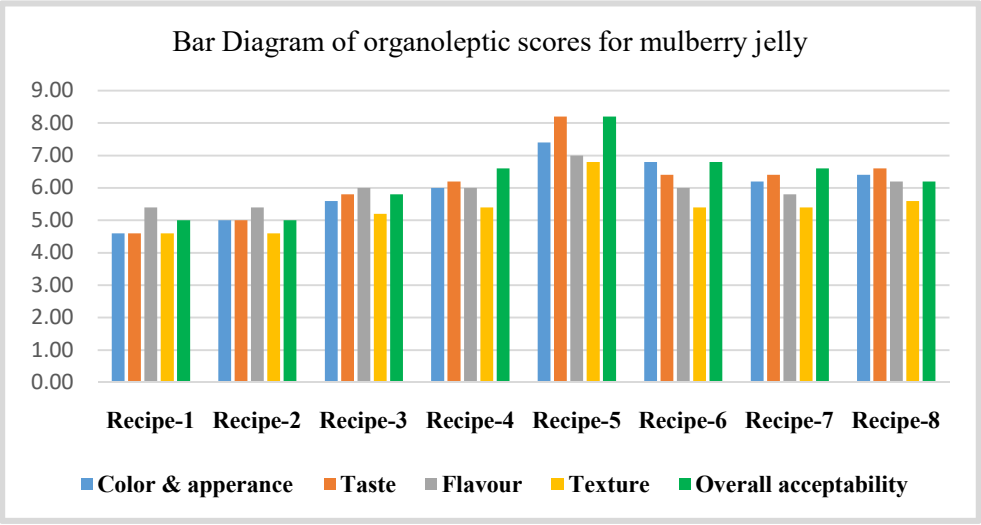

Figure 3: Standardization of recipe for mulberry jelly

The score was maximum for Recipe- 5 (T5): (MFJ + 50% sugar + 0.5% pectin + 0.3% citric acid + 0.1% sodium benzoate and it were 8.20 while the minimum score was recorded in Recipe- 1 (T1). The minimum scores for the first three recipes might be due to the amount of sugar and combination with the pectin percentage. According to the organoleptic score for color & appearance, taste, flavor, texture and overall acceptability the Recipe- 5 (T5) was suggested for mulberry jelly preparation.

Table 1: Nutritional Facts of mulberry jelly

Chemical Properties	Mean ± SD
pH	3.663 ± 0.068
Titrable Acidity (g/100 g as citric acid)	0.208 ± 0.027
Total Soluble Solids (TSS)% m/m, ^{0}Brix	67.903 ± 0.230
Total Carbohydrate%	56.430 ± 0.470
Total Sugar%	41.977 ± 0.438
Vitamin-C, mg/100g	2.323 ± 0.451
Iron (Fe), mg/100g	1.213 ± 0.233
Calcium (Ca), mg/100g	22.630 ± 0.607

According to the final recipe mulberry jelly was prepared and nutritional facts were evaluated (Table 1). pH of jelly was found to be 3.663 ± 0.068 and titrable acidity was 0.208 ± 0.027 g/100g as citric acid. Mulberry jelly was found to contain minerals Fe and Ca at the rates of 1.213 ± 0.233 and 22.630 ± 0.607 mg/100 g respectively. Total carbohydrate content of the jelly was 56.430 ± 0.470 percent and 2.323 ± 0.451 mg/100 g. Vitamin C was also estimated in mulberry jelly (2.323 ± 0.451 mg/100g).

Sauce from mulberry fruits:

Standardization of recipe

The sensory scores for different treatments are presented graphically in Figure 4 on 9 point hedonic scale.

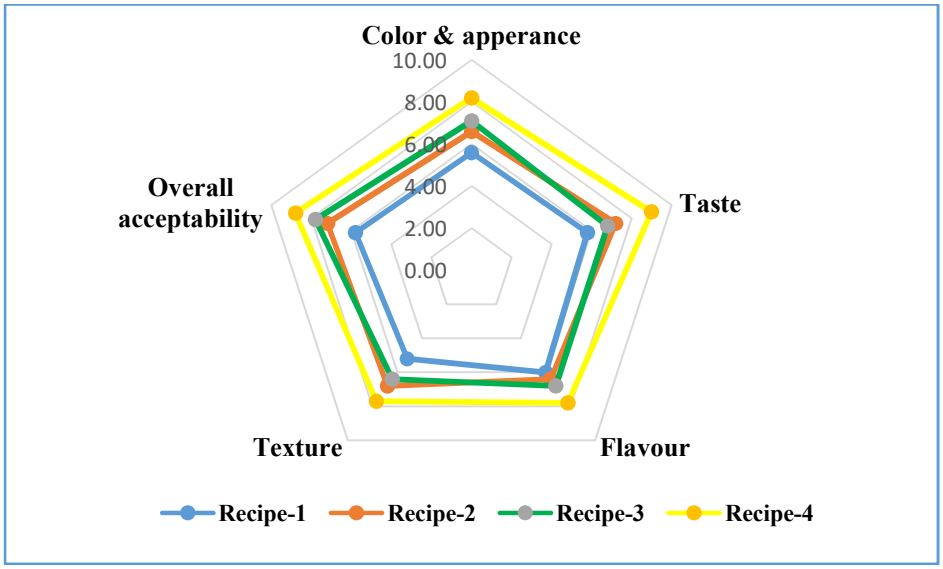

Figure 4: Standardization of the recipe for mulberry sauce

The score was found to be maximum for Recipe- 4 (T4) (MFJ: Water = 80:20 + Other ingredients like salt, sugar, vinegar, citric acid, different spices and 1% sodium benzoate) but, the minimum score was obtained for Recipe- 1 (T1) (MFJ: Water = 50:50 + Other ingredients). Thus, it could be concluded that with the higher concentration of MFJ at the rate of MFJ 80 parts and water 20 parts is the best recipe.

According to the organoleptic score for color & appearance, taste, flavor, texture and overall acceptability the Recipe-4 (T4) was suggested for mulberry sauce formulation.

Table 2: Nutritional Facts of mulberry sauce

Chemical Properties	**Mean ± SD**
pH	3.550 ± 0.082
Titrable Acidity (g/100 g as citric acid)	0.794 ± 0.058
Total Soluble Solids (TSS)% m/m, ^{0}Brix	28.343 ± 0.490
Total Carbohydrate%	53.367 ± 0.367
Total Sugar%	34.790 ± 0.439
Vitamin-C, mg/100g	1.757 ± 0.380
Iron (Fe), mg/100g	1.193 ± 0.091
Calcium (Ca), mg/100g	24.287 ± 0.580

According to the final recipe mulberry sauce was prepared and nutritional facts were evaluated (Table 2). The pH of mulberry sauce was found to be 3.550 ± 0.082 and titrable acidity was 0.794 ± 0.058 g/100g as citric acid. Mulberry sauce was found to contain Iron and Calcium at the rate of 1.193 ± 0.091 and 24.287 ± 0.580 mg/100 g respectively. Total carbohydrate content of the sauce was 53.367 ± 0.367 percent and 1.757 ± 0.380 mg/100 g. Vitamin C was also estimated in mulberry sauce (1.757 ± 0.380 mg/100g).

11.2. Preparation of mulberry tea from mulberry leaf:

The mean nutrient compositions of mulberry leaves at different maturity level are shown in Table 3. The nutritive values showed significant differences among the leaf maturity stages. Highest moisture (77.32%) and crude protein content (18.91%) were obtained in tender leaves and lowest in coarse leaves (69.14%) and (15.40%) respectively. Maximum amount of minerals (11.65%) was found in coarse leaves and minimum in tender leaves (9.81%). The reducing sugar (1.84%), total sugar (3.58%) and starch (13.27) content were observed significantly higher in coarse leaves compared to the other leaf at maturity level. Moreover, it was also found that the moisture and crude protein contents of mulberry leaves were decreased with the increase of leaf maturity level whereas the reducing sugar, total sugar and starch contents were found to be increased with the increase of leaf maturity.

Table 3: Nutrient contents of mulberry leaf at different maturity level (Mean ± SD)

Leaf Type	Moisture%	Crude Fiber%	Mineral%	Crude protein%	Reducing sugar%	Total sugar%	Starch%
Mixture	72.66 ± 1.33	14.13 ± 0.15	10.62 ± 0.47	17.23 ± 0.31	1.73 ± 0.05	3.43 ± 0.29	12.40 ± 0.38
Mid Mature (MTS- 2)	73.72 ± 1.38	13.44 ± 0.52	10.02 ± 0.91	16.26 ± 0.48	1.53 ± 0.20	3.18 ± 0.19	12.03 ± 0.47
Tender (MTS- 3)	77.32 ± 1.01	10.31 ± 0.51	9.84 ± 0.06	18.91 ± 0.59	1.05 ± 0.10	2.34 ± 0.25	10.84 ± 0.26
Coarse	69.14 ± 1.00	15.18 ± 0.69	11.65 ± 0.53	15.40 ± 0.19	1.84 ± 0.07	3.58 ± 0.15	13.27 ± 0.40
Grand Mean	73.21	13.27	10.53	16.95	1.54	3.13	12.13
%CV	0.57	2.18	6.06	4.71	7.69	6.9	3.51

Table 4 shows the quality of mulberry tea samples as for Water extract%, Total ash%, Water soluble ash of total ash%, Alkalinity of water soluble ash% (as KOH), Acid insoluble ash% and Crude fiber%. The soluble matters such as phenolic compounds, alkaloids, amino acids, and many minor soluble substances extracted from the tea samples which determines the quality of the tea is known as water extract. The amount of water extract of tea depends on tea and water ratio, temperature of the tea brew, type, and size of made tea particles.

Table 4: Quality assessment of mulberry tea samples (MTS)

Treatments	Water Extract (% m/m)	Total Ash (% m/m)	Water soluble ash of total ash (% m/m)	Alkalinity of water soluble ash as KOH (% m/m)	Acid Insoluble ash (% m/m)	Crude Fiber (% m/m)
MTS- 1 (Mixture)	22.96 ± 0.06	11.58 ± 0.29	50.11 ± 0.15	3.10 ± 0.03	0.81 ± 0.10	12.86 ± 0.17
MTS- 2 (Mid Mature)	19.01 ± 0.12	10.11 ± 0.04	48.15 ± 0.06	2.89 ± 0.10	0.63 ± 0.05	10.48 ± 0.07
MTS- 3 (Tender)	18.67 ± 0.19	9.83 ± 0.14	50.56 ± 0.33	3.83 ± 0.06	1.07 ± 0.05	8.70 ± 0.20
%CV	0.66	1.79	0.44	2.24	8.45	1.46
Reference Value for Black Tea (as per BSTI Std, BDS 1107, 1984)	**32.00 (Min)**	**4.00-8.00**	**45.00 (Min)**	**1.00-3.00**	**1.00 (Max)**	**16.50 (Max)**
Reference of Test Method	BDS 808:1974, APP-B	BDS 808:1974, APP-C	BDS 808:1974, APP-D	BDS 808:1974, APP-E	BDS 808:1974, APP-F	BDS 808:1974, APP-G

Data are presented as Mean ± Standard Deviation

Water extract % was found to be highest for Mulberry tea sample- 1 (22.96 ± 0.06) and lowest for MTS- 3 (18.67 ± 0.19) but BSTI reference value was 32.00 (min). Total ash content was also found highest for MTS- 1 (11.58% ± 0.29) and lowest for MTS- 3 (9.83% ± 0.14). Ash content of tea is also an important quality parameter. The higher ash content in tea might be due to less moisture content in tea. Total ash content in tea correlates with mineral content of the tea sample as well. The composition of the ash of tea varies somewhat with the age of the leaf because the water soluble potash and phosphoric acid diminishes as the leaves become mature. Total ash content both for MTS- 1 and MTS- 3 was found to be high compared to the reference value of 4.00-8.00 due to containing of high minerals in mulberry leaf. For both the mulberry tea samples water soluble ash content usually indicates an inferior tea which may have been produced using mature leaves. Water soluble ash, of total ash% was found to be similar for MTS- 1 & MTS- 3 but comparably it was lower for MTS- 2 (48.15 ± 0.06). For all the three mulberry tea samples Water soluble ash, of total ash% was found to be slightly high compared to the reference value of black tea (45.00 min). Alkalinity of water soluble ash% (as KOH) was highest for MTS- 3 (3.83 ± 0.06) and lowest for MTS- 2 (2.89 ± 0.10) but for MTS- 1 it was found to be 3.10 ± 0.03 which is very much nearest to the reference value 1.00-3.00. There was a significant difference among the mulberry tea samples in acid insoluble ash% content. The lowest acid insoluble ash% was estimated for MTS- 2 to be 0.63 ± 0.05 and second lowest was for MTS- 1 (0.81 ± 0.10) and MTS- 3 showed the highest acid insoluble ash% (1.07 ± 0.05). The acid insoluble ash% value of MTS- 1 and MTS- 2 was acceptable according to the reference value (1.00 max) of BSTI for Black Tea. Crude fiber% was found lowest for MTS- 3 (8.70 ± 0.20) due to less maturity and 12.86 ± 0.17 for MTS-1 (mixture leaves). For all the three mulberry tea samples the crude fiber% was acceptable according to the reference value of crude fiber content for black tea (16.00 max). Crude fiber content in tea is an important quality parameter as the low fiber content in tea samples may be attributed to the use of younger tea leaves to manufacture tea. High fiber content in tea samples may be due to impurities like stems during processing as the roll breaking and stalks removal step is absent during manufacturing process. In addition to this, crushing,

tearing, and curling process also destroy the leaf structure that might have an effect on fiber content but in the processing of mulberry tea samples all the process step was followed by manually.

Table 5: Comparison of DPPH free radical inhibition % of mulberry tea samples with commercial teas

Sample Name	Aqueous decoction of 2.0 gm tea dust DPPH free radical inhibition % (Mean ± SD)			% CV
	T-1	T-2	T-3	
1. Green Tea (Kazi & Kazi)	50.73 ± 0.29	68.54 ± 0.55	73.25 ± 0.24	0.6
2. Tulsi Tea	36.34 ± 0.88	56.75 ± 0.34	64.88 ± 0.24	1.07
3. Green Tea (Rings Herbs)	65.77 ± 0.40	74.78 ± 0.49	82.20 ± 0.33	0.55
4. Seylon	84.39 ± 0.46	90.89 ± 0.37	92.52 ± 0.52	0.51
5. Taaza	63.33 ± 0.49	70.65 ± 0.51	77.64 ± 0.37	0.65
6. Mirzapur Tea	39.67± 0.49	68.94 ± 0.44	84.47 ± 0.25	0.63
7. Jute Tee	33.41± 0.35	40.24 ± 0.27	50.00 ± 0.71	1.18
8. MTS-1	44.47± 0.36	46.42 ± 0.42	54.88 ± 0.65	1.01
9. MTS-2	12.85± 0.61	15.04 ± 0.33	30.33 ± 0.27	2.22
10. MTS-3	15.37± 0.31	21.63 ± 0.26	22.28 ± 0.31	1.5

Table 5 shows the comparison of DPPH free radical inhibition % of mulberry tea samples with the commercial teas. The methanolic tea extracts had strong antioxidant activity against all the free radicals investigated. The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of observations. In case of treatment T-1 (100 µl) among the commercial tea brands highest DPPH scavenging activity was 84.39% for Seylon tea brand and the lowest was for Jute tea (33.41%) followed by 36.34% for Tulsi tea. But, in case of mulberry tea samples (MTS) it was shown to be 44.47%, 12.85% and 15.37% for MTS- 1, MTS- 2 and MTS- 3 respectively. Among the mulberry tea samples MTS- 1 showed the highest DPPH free radical scavenging activity (44.47%) which was higher than Jute tea and Tulsi tea. With the increase of the concentration of the treatments T- 2, and T- 3 (200 µL and 400 µL respectively) all the tea extracts showed significant increasing trend in free radical inhibition%.

Table 6: Comparison of total phenolic and total flavonoids contents of mulberry tea samples with commercial teas (Data are presented as Mean ± SD)

Sample Name	Total Phenolic contents (mg GAE/g of tea dust)	Total Flavonoids (mg CatE/g of tea dust)
1. Green Tea (Kazi & Kazi)	45.63 ± 0.23	72.66 ± 0.19
2. Tulsi Tea	24.98 ± 0.26	19.65 ± 0.27
3. Green Tea (Rings Herbs)	42.21 ± 0.23	52.25 ± 0.09
4. Seylon	36.79 ± 0.16	38.78 ± 0.19
5. Taaza	33.08 ± 0.07	30.77 ± 0.23
6. Mirzapur Tea	35.26 ± 0.20	44.44 ± 0.36
7. Jute Tee	20.84 ± 0.17	16.81 ± 0.17
8. MTS-1	28.09 ± 0.20	28.01 ± 0.39
9. MTS-2	19.11 ± 0.11	24.64 ± 0.25
10. MTS-3	17.17 ± 0.13	21.25 ± 0.25
Total Grand Mean	30.316	34.927
% CV	0.29	0.34

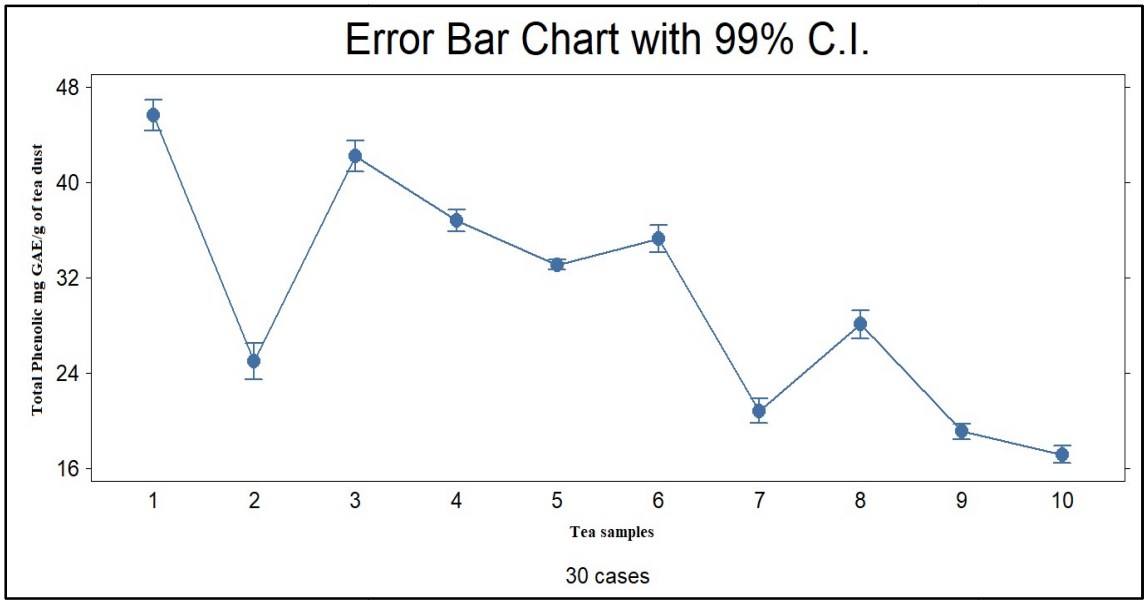

Figure 5: Comparative total phenolic contents (mg GAE/g) of 10 different tea samples

Table 6 shows the comparison of total phenolic and total flavonoids contents of mulberry tea samples with commercial teas, and Figure 5 shows the comparative total phenolic contents (mg GAE/g) of 10 different tea samples. Phenolic compounds have redox properties which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. In case of familiar tea brands total phenolic content of the methanolic extracts of different tea dust were estimated from the calibration curve (R^2=0.9908) and the highest value was found to 45.63 ± 0.23 for Green tea (kazi and kazi) and the lowest value was 24.98 ± 0.26 for Tulsi tea (mg galic acid equivalents/g). In case of mulberry tea samples (MTS) total phenolic content was found highest for MTS-1 (28.09 ± 0.20) which is higher than Tulsi tea (24.98 ± 0.26) and Jute tea (20.84 ± 0.17) and lowest total phenolic content was found for MTS- 3 (17.17 ± 0.13).

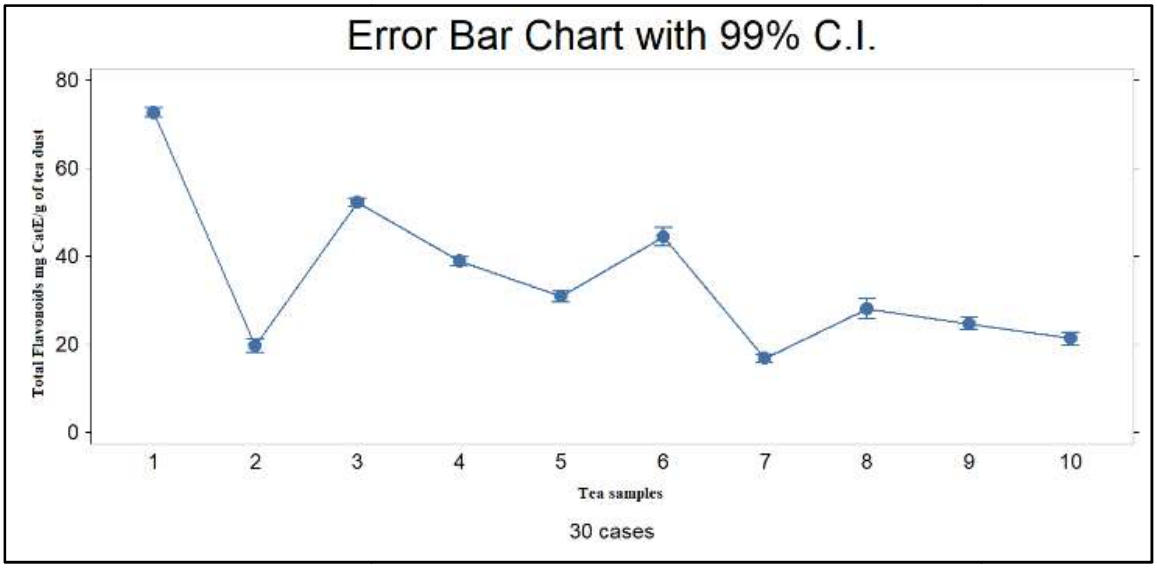

Figure 6: Comparative total flavonoids contents (mg CatE/g) of 10 different tea samples

Figure 6 shows the comparative total flavonoids contents (mg CatE/g) of 10 different tea samples. Total Flavonoids content of the methanolic extracts of different tea dust were also estimated from the calibration curve (R^2=0.9991). As shown in Table 6 above, the highest value for total flavonoids contents was found 72.66 ± 0.19 for Green Tea (Kazi and Kazi) and the lowest value was 16.81 ± 0.17 for Jute tea (mg catechineequivalants/g). For mulberry tea samples (MTS) total flavonoids content was found highest for MTS-1 (28.01 ± 0.39) which is greater than Tulsi tea and Jute tea 19.65 ± 0.27 and 16.81 ± 0.17 respectively and very much closest to Taaza (30.77 ± 0.23). The lowest total flavonoids content was found 21.25 ± 0.25 for MTS- 3.

11.3. Extraction of oil from silkworm pupae and its fatty acid profile analysis:

Table 7: Nutrient contents of pupae powder

Types of Pupae	Moisture (%)	Crude Protein (%)	Oil. (%)	Ash (%)
Female	7.19 NS	43.3**	26.11***	7.04**
Male	6.94 NS	40.87**	21.44***	6.14**
SEm (±)	0.08	0.27	0.21	0.21
% CV	2.04	1.12	1.55	3.41

*Level of significance, ** Significant at P<0.05, *** at P<0.01 and NS= Not significant*

Table 8: Nutrient contents of pupae residue after oil extraction

Types of Pupae	Moisture (%)	Crude Protein (%)	Oil (%)	Ash (%)
Female	4.89 NS	60.56**	0.57**	11.21 NS
Male	4.65 NS	56.28**	0.41**	10.39 NS
SEm (±)	0.11	0.50	0.03	0.30
% CV	3.99	1.50	11.63	4.80

*Level of significance, ** Significant at P<0.05, *** at P<0.01 and NS= Not significant*

The chemical constituents of pupae powder after and before oil extraction were analyzed and are presented in Table 7 and Table 8. Crude protein content was calculated by Kjhaldahl Method and conversion factor was 6.25. Crude protein percentage was 43.3 and 40.87 for female and male pupae respectively. Oil content % was also 26.11 and 21.44 for female and male pupae respectively. But after oil extraction crude protein % was significantly increased in the residue of the pupae powder; 60.56 and 56.28 in case of female and male pupae respectively. From the above tables it appears that there are significant differences of crude protein and oil content in female and male pupae. For pupae oil extraction from silkworm pupae, female pupae gave the higher amount of oil compared to the male pupae.

Table 9: Relative extractability of oil from pupae with different solvents

Types of Pupae	Hot Condition		Cold Condition	
	Solvent-1	Solvent-2	Solvent-1	Solvent-2
Female	25.11***	21.91***	20.81***	17.98 NS
Male	20.94***	17.98***	17.73***	16.69 NS
SEm (±)	0.14	0.27	0.22	0.49
% CV	1.04	2.36	1.96	4.85

*Solvent-1: Petroleum Ether, Solvent-2: n-Hexane, ** Significant at P<0.05, *** at P<0.01 and NS= Not significant.*

The relative extractability percentage of pupae oil from silkworm pupae with two different solvent viz; solvent-1 refers Petroleum Ether and solvent-2 refers n-Hexane are shown in the Table 9. The data indicate that hot extraction gave relatively more oil that of cold extraction both for female and male pupae. The highest extractability of oil was observed with petroleum ether (26.11%) in case of female pupae followed by n-Hexane (21.91%) with hot condition. The lowest extractability was observed with n-Hexane (17.73%) in case of male pupae with cold condition. Female pupae with petroleum ether solvent showed high performance in hot condition for relative extractability of pupae oil from silkworm pupae.

Table 10: Fatty acid profile analysis of pupae oil extracted with two different solvents

Solvents	Fatty acid concentration % with different solvent							
	Palmitic Acid	**Stearic Acid**	**Linoleic Acid**	**Oleic Acid**	**Palmitoleic Acid**	**Margaric Acid**	**Heneicosanoic Acid**	**9, 12, 15 Octadecatrienoic Acid**
Pet. Ether	17.504	20.435	15.126	44.154	0.8533	0.414	0.4103	0.242
n-Hexane	33.72	22.014	1.388	43.466	ND	ND	ND	ND
SEm (±)	0.4536	0.4795	0.1688	0.4132	0.0568	0.0797	0.0403	0.0173
% CV	3.07	3.91	3.54	1.63	23.04	66.65	34.03	24.81
LS	***	NS	***	NS	***	**	***	***

*LS= Level of significance, ** Significant at P<0.05, *** at P<0.01 and NS= Not significant*

Table 10 shows a significant difference of fatty acid content except stearic acid & Oleic acid for two different solvents viz., Petroleum Ether and n-Hexane. According to the table-9 above, petroleum ether as solvent-1 showed highest performance for relative extractability and from the table 10 it could be seen that pupae oil extracted with n-Hexane (Solvent-2) failed to identify four fatty acids viz., Palmitoleic acid, Margaric acid, Heneicosanoic acid and 9, 12, 15 Octadecatrienoic acid.

As shown in Table 10, silkworm pupae oil would be a great source of four different fatty acids viz., Palmitic acid (17.5% and 33.7%, extracted with Pet. Ether and n-Hexane respectively), Stearic acid (20.4% and 22%, extracted with Pet. Ether and n-Hexane respectively), Linoleic acid (15% and 1.4% extracted, with Pet. Ether and n-Hexane respectively) and Oleic acid (44% and 43.5%, extracted with Pet. Ether and n-Hexane respectively). Palmitic acid can act as an emollient, softening the skin and help retain moisture by forming an occlusive layer. According to a Korean study published in a 2010 edition of the "Journal of Medicinal Food," palmitic acid does possess antioxidant properties. The antioxidants help prevent free radical damage to maintain youthful radiant skin. Two main functions of palmitic acid are to act as an emulsifier and surfactant. The low surface tension of palmitic acid allows water to combine with the oil and dirt molecules and wash them away. As a result, palmitic acid helps to remove dirt, sweat and excess sebum from the skin and hair. This makes it a useful ingredient in cleansers, body washes, shampoos and bar soaps. Palmitic acid helps to combine the water and oil phase of the formulation, enabling the formation of a cream, lotion or any liquid cosmetic. Silkworm pupae oil showed Palmitic acid 17.504% and 33.72% for solvent-1 and solvent-2 respectively.

Stearic acid can be used in a variety of cosmetic creams, cakes, soaps and pastes. Stearic acid used as Surfactant cleansing agent and Surfactant emulsifying agent in cosmetic and personal care products. It can be used as lubricant, emulsifier. Stearic acid can be used as intermediate, Lubricants, defoamers in food and in beverage. Stearic acid is widely used as intermediate in Pharmaceutical. Silkworm pupae oil showed highest stearic acid content 22.014% for n-Hexane and 20.435% for Pet. Ehter solvent. There

was a significant difference (P<0.01) in Linoleic acid content 15.126% for Pet. Ether but 1.388% for n-Hexane solvent.

Oleic acid have a lot of beauty benefits such as; act as moisturizing and anti-aging properties, boosts hair growth and prevents dry scalp and dandruff, etc. Oleic acid also have some wide use in pharmaceuticals industries due to have some health benefits such as boosts the immune system, fights free radical damage, anti-inflammatory effects, supports cardiovascular health & respiratory health, helps in weight loss and in regulating blood sugar, etc. Among different fatty acid content in silkworm pupae oil, highest amount fatty acid content was found as Oleic Acid. Highest oleic acid content was found to be 44.154 % for Petroleum Ether solvents and 43.466% for n-Hexane solvent.

Figure 7 shows the photos of final products (mulberry jelly, mulberry sauce, mulberry tea and silkworm pupa oil) of the project.

Figure 7: Photo view showing final products (mulberry jelly, mulberry sauce, mulberry tea and silkworm pupa oil) of the project.

12. Research highlight/findings:

- Nutritionally enriched mulberry jelly was prepared from mulberry fruits by adding with mulberry fruit juice (MFJ) sugar 50%, pectin 0.5%, citric acid 0.3% and 0.1% sodium benzoate.
- Nutritional analysis of the prepared jelly revealed the pH to be 3.663 and titrable acidity was 0.208 g/100g as citric acid. Mulberry jelly was found to contain Fe and Ca at the rates of 1.213 and 22.630 mg/100 g respectively. Total carbohydrate content of the jelly was 56.430 percent and 2.323 mg/100 g. Vitamin C content was estimated to be 2.323 mg/100g.
- Mulberry fruit juice (MFJ) and Water ratio of 80:20 with other ingredients was found to be the best recipe for mulberry sauce.
- Nutritional facts of mulberry sauce revealed the pH to be 3.550 and titrable acidity was 0.79 g/100g as citric acid. Mulberry sauce was found to contain Iron and Calcium at the rate of 1.193 and 24.287 mg/100 g respectively. Total carbohydrate content of the sauce was 53.367 percent and 1.757 mg/100 g. Vitamin C content was estimated to be 1.757 mg/100g.
- To get best quality mulberry tea it was suggested to use mixture of mulberry leaves (tender, mid-mature and coarse).
- Pupae oil from silkworm pupae was extracted and its fatty acid profile analyzed. Crude protein content was 43.3 and 40.87% for female and male pupae respectively. Oil content was also 26.11 and 21.44% for female and male pupae respectively. But after oil extraction crude protein content was significantly increased in the residue of the pupae powder that was 60.56% and 56.28% in case of female and male pupae respectively.
- Silkworm pupae oil would be a great source of four different fatty acids viz., Palmitic acid (17.5% and 33.7%), Stearic acid (20.4% and 22%), Linoleic acid (15% and 1.4%) and Oleic acid (44% and 43.5%) when extracted with Pet. Ether and n-Hexane respectively.

B. Implementation Position

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	a) Laptop-01 b) Camera-01	60000.00 25000.00	a) Laptop-01 b) Camera-01	59800.00 24800.00	100% Achieved.
(b) Lab &field equipment	a) Fiber Analyzer Machine-01 b) Micro Oven-01 c) Freezer-01	500000.00 50000.00 150000.00	a) Fiber Analyzer Machine-01 b) Micro Oven-01 c) Freezer-01	499400.00 48500.00 149400.00	100% Achieved.
(c) Other capital items					

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: N/A

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training					
(b) Workshop					

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	132129	132129	132129	0	100	
B. Field research/lab expenses and supplies	2906626	2790368	2790368	0	100	
C. Operating expenses	299950	299950	298234	1716	99.43	Bank account maintenance cost from October 19 to closing date.
D. Vehicle hire and fuel, oil & maintenance	168000	139440	139440	0	100	
E. Training/workshop/seminar etc.	0	0	0	0	0	
F. Publications and printing	274128	227084	149128	77956	65.67	PCR cost which already refunded.
G. Miscellaneous	49600	49120	44800	4320	91.21	PCR Tender Committee Honorarium cost which already refunded.
H. Capital expenses	781900	781900	781900	0	100	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To prepare Jelly and Sauce from mulberry fruits.	➢ Maintenance of experimental field for mulberry fruits. ➢ Harvesting and processing of mulberry fruits. ➢ Appropriate recipe development for mulberry jelly and sauce preparation. ➢ Nutritional analysis of developed products.	➢ Mulberry jelly & sauce developed. ➢ Mulberry jelly was found to contain Fe and Ca at the rates of 1.213 and 22.630 mg/100 g respectively. Total carbohydrate content of the jelly was 56.430 percent and 2.323 mg/100 g. Vitamin C content was estimated to be 2.323 mg/100g and the pH was 3.663 with the titrable acidity of 0.208 g/100g as citric acid. ➢ Mulberry sauce was found to contain Iron and Calcium at the rate of 1.193 and 24.287 mg/100 g respectively. Total carbohydrate content of the sauce was 53.367 percent and 1.757 mg/100 g. Vitamin C content was estimated to be 1.757 mg/100g and the pH was 3.550 with titrable acidity of 0.79 g/100g as citric acid.	It is hoped that mulberry jelly and sauce developed from mulberry fruits and mulberry tea developed from mulberry leaves will have commercial value as a value added product of sericulture industry. Silkworm pupae oil will also be able to earn a great value in making cosmetics and pharmaceutical products.
To prepare mulberry tea from mulberry leaves.	➢ Nutritional analysis of mulberry leaf with different maturity level ➢ Plucking of mulberry leaves and processing for tea preparation ➢ Quality analysis and anti-oxidant properties estimation	➢ Considering all the parameters of nutrient contents and anti-oxidant properties it was suggested to use mixture of mulberry leaves (tender, mid mature and coarse) to get best quality mulberry tea.	
To extract oil form silkworm pupae and study on its fatty acid profile.	➢ Rearing of silkworm for silkworm pupae ➢ Sex separation of silkworm pupae. ➢ Preparation of pupae powder ➢ Extraction of oil from pupae ➢ Fatty acid profile analysis	➢ Analysis of silkworm pupae oil revealed to contain crude protein at the rate of 43.3% and 40.87% for female and male pupae respectively. Oil content was 26.11% and 21.44% for female and male pupae respectively. After oil extraction crude protein in the residue pupae powder was 60.56% and 56.28% for female and male pupae respectively. ➢ Silkworm pupae oil was found to contain Palmitic acid (17.5% and 33.7%), Stearic acid (20.4% and 22%), Linoleic acid (15%	

		and 1.4%) and Oleic acid (44% and 43.5%) when extracted with Pet. Ether and n-Hexane respectively.	

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.			
Journal publication			
Information development			
Other publications, if any			

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

Mulberry jelly and sauce form mulberry fruits.
Mulberry tea from mulberry leaves.
Extraction of oil from silkworm pupae.

ii. **Generation of new knowledge that help in developing more technology in future**

The knowledge generated in relation to nutritional values of mulberry leaves, fruits and silkworm and nutritional facts of mulberry jelly, sauce and mulberry tea will help taking more research for further improvement of the products.

iii. Technology transferred that help increased agricultural productivity and farmers' income

Adoption of the techniques of preparing mulberry jelly and sauce from mulberry fruits, tea from mulberry leaves and extraction of pupae oil from silkworm pupae for commercial use will help farmers to increase their income.

iv. Policy Support

None

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring (description & output of consultation meeting, monitoring workshops/seminars etc.):

Not done

ii) Field Monitoring (time & No. of visit, Team visit and output):

Team Visit	Time	No. of Visit	Output
PIU-BARC, NATP-2	-	02	Observations and valuable suggestions
Internal Monitoring by Director (BSRTI)	-	02	Observations and valuable suggestions
Internal Monitoring by DG (BSDB)	-	01	Observations and valuable suggestions
Other Visitors: Professors and students from the Dept. of Chemistry, Organic Branch	-	01	Observations and valuable suggestions

H. Lesson Learned (if any)

- Time was too short for completion of the project.

I. Challenges (if any)

- It was a big challenge to keep pace with time due to short duration of the project.
- Complex fund disbursement process was a barrier against smooth running of the research work.

Signature of the Principal Investigator
Date …….
Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal