DETERMINATION OF PHENOLICS, FLAVONOIDS AND ANTHOCYANINS IN PIGMENTED AND NON-PIGMENTED MAIZE (*Zea mays L.*) IN BANGLADESH

ISRAT JAHAN PREETY

DEPARTMENT OF BIOCHEMISTRY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

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BY

ISRAT JAHAN PREETY

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Approved by:

Supervisor	**Co-supervisor**
Dr. Kamal Uddin Ahmed	**Dr. Ashrafi Hossain**
Professor, Dept. of Biochemistry	**Professor, Dept. of Biochemistry**

Dr. Ashrafi Hossain
Chairman, Department of Biochemistry
Chairman
Examination Committee

Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207.
Bangladesh.

Ref **Date :**

CERTIFICATE

This is to certify that thesis entitled, ***"DETERMINATION OF PHENOLICS, FLAVONOIDS AND ANTHOCYANINS IN PIGMENTED AND NON-PIGMENTED MAIZE (Zea mays L.) IN BANGLADESH"*** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE in BIOCHEMISTRY,*** embodies the result of a piece of bona fide research work carried out by ***ISRAT JAHAN PREETY,*** Registration No. ***19-10119*** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

.....

Dated:
Place: Dhaka, Bangladesh

Dr. Kamal Uddin Ahmed
Professor,
Department of Biochemistry

Annexure IV. Some commonly used Abbreviations

Full Word	Abbreviation
Aluminum chloride	$AlCl_3$
and others (at elli)	*et al.*
Association of Official Analytical Chemist	AOAC
Bangladesh Agriculture Research Institute	BARI
Catechin equivalents	CE
Carbon	C
Cyanidin-3- glucoside equivalents	C3G
Degree Celsius (Centigrade)	^oC
Dry matter	d.m.
Dry weight	d.w.
Ferulic acid equivalents	FAE
Folin & Ciocalteu's Phenol reagent	FCR
Food and Agricultural Organization	FAO
Gallic acid equivalents	GE
Gram	g
Hour	hr
Hydrogen ion conc.	pH
Hydrochloric Acid	HCl
Kilogram	kg
Least significant difference	LSD
Liter	L
Magnesium	Mg
Manganese	Mn
Microgram	µg
Micromole	µmol
Micron	um/µ
mill equivalent	meq
Milligram	mg
Milliliter	mL
Millimicron	mu/mµ
Minute	min.
Molar	M
Molecular weight	MW
Normal	N
Normality	N
Parts per Million	ppm
Percentage	%
Phosphorus	P
Potassium	K
Potassium Chloride	KCl
Randomized complete block design	RCB
Sodium	Na
Sodium Carbonate	Na_2CO_3
Sodium hydroxide	NaOH
Sodium Nitrate	$NaNO_2$

Standard Deviation	SD
Total Anthocyanin Content	TAC
Total Phenolic Content	TPC

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The Author

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DETERMINATION OF PHENOLICS, FLAVONOIDS AND ANTHOCYANINS IN PIGMENTED AND NON-PIGMENTED MAIZE (*Zea mays L.*) IN BANGLADESH

ABSTRACT

Maize is one of the most diverse grain crops and most widely cultivated cereals in the world. Multicolored maize such as yellow, white, red, and purple maize are produced across the Bangladesh. The present study was conducted to observe the amounts of pigments responsible for pericarp color such as phenolics, flavonoids and anthocyanin in six different colored maize varieties using UV-spectrophotometer as a tool. The research was carried out during the time form December 2020 to March 2021 at the Biochemistry laboratory of Department of Biochemistry, and Agro-Environmental Chemistry Laboratory of Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka. In the study, the contents of methanol soluble or free phenolics, acidic methanol soluble or bound phenolics were determined in the whole kernels of six different pigmented and non-pigmented maize such as-yellow maize, red maize, white maize, purple maize, mixed colored maize, deep red maize. In this study, it was observed that the SAU purple maize was rich in phenolics content. It contained 105.82 ± 8.3 mg FAE/100 g sample free phenolics; 165.05 ± 7.5 mg FAE/100 g sample of bound phenolics and 270.87 ± 15.80 mg FAE/100 g sample of total phenolics. SAU purple maize contained the highest flavonoids (76.49 ± 9.5 mg CE/100 g dry weight) and as well as anthocyanins (68.58 ± 5.3 C3G equv./100 f of dry weight sample) among the pigmented and non-pigmented maize varieties. On the other hand, white maize a non-pigmented maize performed the lowest amount of phenolics and flavonoid content. White maize had the amount of 30.55 ± 1.61 mg FAE /100 g of flour in free phenolics, 46.85 ± 4.4 mg FAE /100 g of flour in bound phenolics, and 77.40 ± 5.7 mg FAE /100g of total phenolics content. Total flavonoids content in white maize was 8.82 mg CE /100 g sample dry basis. Yellow maize performed the lowest for total anthocyanin contents 4.7 ± 1.1 mg C3G/100g of sample dry basis. Percentage contribution of insoluble phenolics to total phenolics, and flavonoids to insoluble phenolics was great in the SAU purple maize. Overall, pigmented maize varieties are rich in health beneficial phytochemicals. SAU purple maize showed the great promise for its future usage as human food or as food ingredients. Use of such colored maize in food preparation could add additional immunity to its consumers.

CHAPTER I

INTRODUCTION

Cereal grains are the storage organ that comprise proteins, carbohydrates, vitamins, minerals, and oils required for metabolic activity during growth and development of a new plant (Niroula *et al.,2019*). Maize kernel is the largest storage seed in the cereals. Maize kernels come with pigmented and non-pigmented pericarps. Pigmentation of the pericarp is due to presence of various colored compounds in various content. Major pigments or color compounds present in maize pericarp are different types of phenolics, flavonoids, anthocyanins, carotenoids etc. They are also called phytochemicals. Among all color compounds, phenolics are abundant in our diet includes grains (maize, rice, wheat, millets etc.), fruits and vegetables (Adom *et al*., 2002; Pandey *et al.*,2009). Phenolics are also present in medicinal plants. Several researches have shown that consumption of phenolics rich diet is health beneficial. They are anticancerous, cardioprotective, anti-inflammatory, neurodegenerative, hypercholesterolemia, hyperglycemia, hyperlipidemia, and showed antitumor activity or have reduced oxidative stress (Adom *et al*., 2002; Miguel *et al.*,2013; Wu *et al.*,2016;). Furthermore, phenolics might modulate the activity of a wide range of enzymes and cell receptors. In this way, in addition to having antioxidant properties, phenolics have several other specific biological actions that are as yet poorly understood (Manach *et al.*,2004).

Phenolics are a group of compounds with phenolic structural features abandon in nature. The term polyphenol is also used as a collective term for several sub-groups of phenolic compounds. More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common

intermediate, phenylalanine, or a close precursor, shikimic acid (Pandey *et al.*, 2009; Tsao, 2010). Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linked with other phenol may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another (Tsao, 2010).

The main classes of polyphenols might include phenolic acids, flavonoids, stilbenes and lignans. Figure 1 illustrates the different groups of polyphenols with examples and their chemical structures.

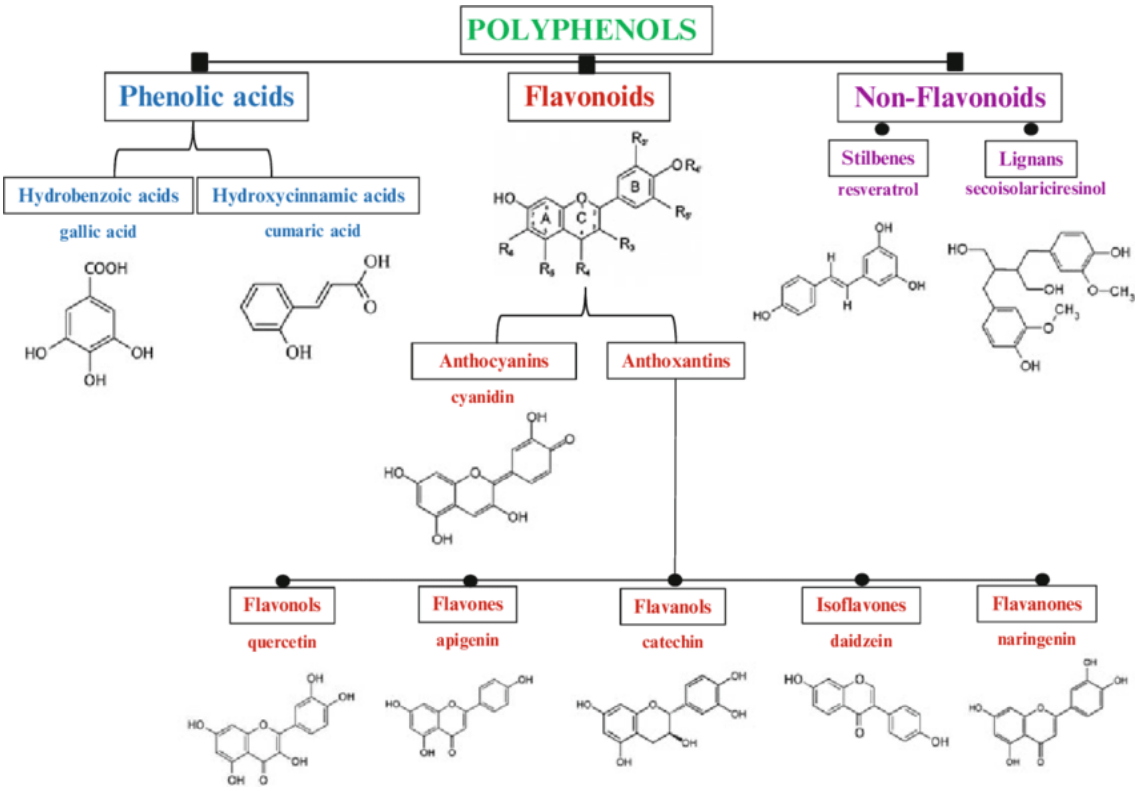

Figure 1: Classification of phenolics (Rigacci *et al.*, 2015)

Phenolic acids are non-flavonoid polyphenolic compounds. Phenolic acids might further be classified into two types, Benzoic acid and cinnamic acid derivative based on C_1-C_6 and C_3-C_6 backbones. Gallic acid, Vanillic acid are some example of benzoic acids, and ferulic acid and sinapic acid are some examples of cinnamic acids (Tsao., 2010).

Flavonoids, a group of polyphenols are the most abundant in nature which has more than 5000 different types. The main subclasses of flavonoids include the flavones, flavonols, flavanones, catechins or flavanols, anthocyanidins and isoflavones. Figure 2 illustrates basic skeleton of flavonoids and its main subclasses with structures. Most of the flavonoids without proanthocyanidins are available in plants with attached sugars such as aglycones (Prior *et al*., 2006).

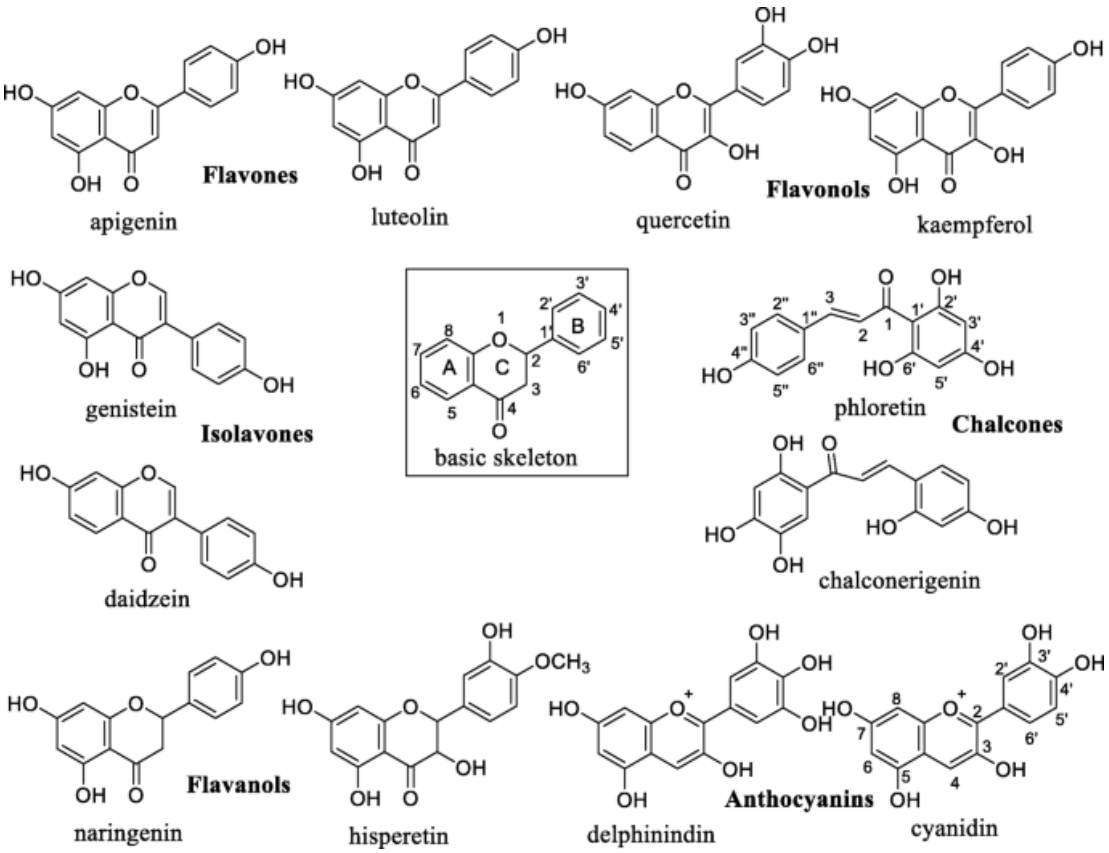

Figure 2. Classification of flavonoids (Habila *et al.*, 2020)

The chemical structure of flavonoids varies according to the hydroxylation pattern, conjugation between aromatic rings, glycosidic moieties, methoxy groups and other substituents (Santos et al., 2017). Flavonoids contain C_6-C_3-C_6 basic structure where it has two C_6 units (ring A and ring B) are of phenolic nature. The wide majority of the flavonoids have the ring B attached to the C_2 position of ring C. In few flavonoids such as isoflavones and neoflavonoids, ring B is attached at the C_3 and C_4 position of ring C (Rong et al., 2010). Biological activities may vary on the basis of structural differences and the glycosylation pattern. Flavonoids has the widest color range from pale-yellow to blue pigments that are responsible for coloring of most flowers, fruits and seeds. Flavonols are the most universal flavonoids of nutritional interest in foods and among them, quercetin and kaempferol are abandon in nature (Rong *et al.,* 2010; Santos *et al.*, 2017). The major types of flavonoids identified in cereal grains are flavonols, anthocyanins and proanthocyanidins.

Anthocyanins belongs to subclass flavonoids and the family of polyphenolics are the most striking members of the secondary plant metabolite. Most of the anthocyanins are water-soluble pigmented phenolics in nature which results in red, purple and blue colors in fruits, vegetables, cereal grains and flowers (Bueno *et al.,* 2012). All anthocyanins share a basic structure; consist of flavylium ion with two aromatic rings linked by a three- carbon heterocyclic ring that contains oxygen (Francavilla *et al.*,2020). The core structure of anthocyanins is anthocyanidins found in glycosidic form. Over 23 anthocyanidins and 500 various anthocyanins have been identified in plants. Chemically, anthocyanins are glycosilate polyhydroxy derivatives of 2-phenylbenzopyrilium containing two benzyl rings. The red, blue, purple colors of various fruits, vegetables, flowers are because of the presence of anthocyanins (Giusti

et al.,2010). They are the major contributor in grain pigmentation and ensure protection of grains against UV-B radiation. Glycosidic derivatives including cyanidin-3-glucoside, penidin-3-glucoside and delphinidin-3-glucoside which are the major types of flavonols and anthocyanins present in pigmented grains (Liu *et al.*, 2013).

The six major anthocyanins are cyanidin, delphinidin, malvidin, pelargonidin, petunidin and peonidin. Figure 3 illustrates the basic structure of anthocyanins. About 50% of anthocyanins present in fruits and cereals are cyanidin derivatives, as followed by pelargonidin (12%), delphinidin (12%), peonidin (12%), petunidin (7%) and malvidin (7%). The conjugated bonds of anthocyanidins develops molecule's color (Iris j joye *et al.*, 2020).

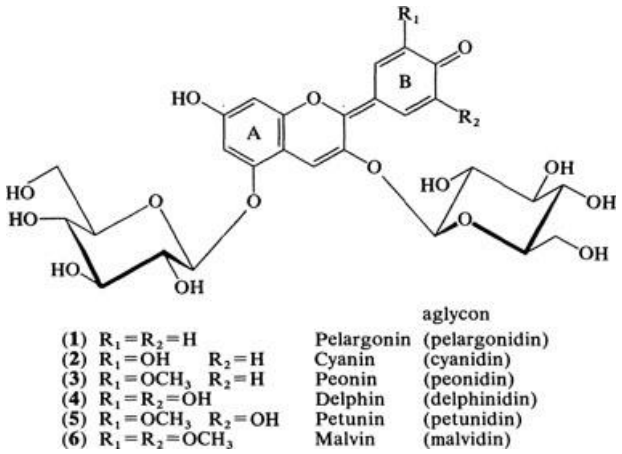

Figure 3. Structure of anthocyanins (Goto, 1987)

Wide range of phenolics, flavonoids, and anthocyanins are present in cereals. Maize is such a grain which can be consumed in whole form. It comes in different colors due to presence of various polyphenols in different amount (Liu *et al.*, 2013; Rooney *et al.*,2007). Polyphenols present in maize is listed in the Table 1.

Table 1. Phenolics compound reported in cereal grains (maize)

Phenolic acids (Hydroxybenzoic and Hydroxycinnamic acids)	**References**
Protocatechuic, P- hydroxybenzoic, Gentisic, Syringic, cinnamic, Ferulic, *m* coumaric	Hahn *et al*., 1983; Gous, 1989
Flavonoids	
Kaempferol, Quercetin, Leucocyanidin, Leucopelargonidin,	Liu *et al.*, 2013
Anthocyanins	
Cyanidin 3- galactoside, Cyanidin 3- glucoside, Cyanidin 3- rutinoside, Pelargonidin 3- glycoside Pelargonidin glucosides Peonidin 3- glucoside	Rooney *et al.*, 2007

In Bangladesh, maize is used as flour, grits, semolina at home scale. Corn on cob (roasted) as street food, and corn starch and syrup are used in food, paper, or medicine industries. However, there was increasing annual demand for maize hereafter increasing production of maize was observed in 2018-19 (CYMMIT, Ali *et al,*.2008; BBS, 2019).

Several maize varieties are released by BARI to meet the current demand of maize. Non-government organizations and agro-seed industries are promoting maize in Bangladesh to meet the current demand (BARI Annual report 2019-2020). Yellow or white maize are usually cultivated and utilized in various purposes. There are some pigmented or non-pigmented indigenous varieties which are grown by local farmers as food. Consumption of such pigmented maize along with yellow or white maize might have health benefits for the presence of bioactive polyphenols. Limited researches focused on phenolics content and composition in pigmented and non-pigmented maize which are consumed by Bangladeshi population. Evaluation of phenolics in such maize could explore its potentiality for preparation of various foods. Therefore, the present research aims to study the phenolics, flavonoids and anthocyanins in some pigmented and non-pigmented maize. In order to fulfill the above mentioned aim the following objectives have been undertaken

1. To determine the methanol soluble, acidic methanol soluble and total polyphenols in pigmented and non-pigmented whole grain maize.
2. To determine total flavonoids content in whole grain maize.
3. To determine anthocyanin content in whole grain maize.

CHAPTER II

REVIEW OF LITERATURE

Maize is consumed all over the world as staple food for human. White maize has maximum use is starch production in industries. The yellow maize is the common maize consumed in various form worldwide. Besides these two main types there are various pigmented maize used in traditional cuisine all over the world. Pigmented maize is promising due to presence of phytochemicals which have several health beneficial properties. Among various phytochemicals phenolics are the major types present in maize. Content of phenolics, flavonoids and anthocyanins might vary in maize due to varietal difference, agro-ecological conditions or even maturity stages of maize used in food preparation.

Zilic, *et al.* (2012) reported the content of total phenolics, free, conjugated and insoluble bound phenolics respectively in whole kernels of 10 genotypes of different colored maize samples collected from Mexico, United states, France, Serbia, and Nederland. The samples were grown in the field of Maize Research Institute (Belgrade, Serbia) following RCB design with two replications. Total phenolics in white, lemon yellow, yellow and orange maize ranged from 5227.1 to 5811.2 GAE/kg d.m. The light blue and dark blue genotypes had 10528.8 ± 58.8 mg GAE/kg d.m. and 7352.5 ± 498.5 mg GAE/kg d.m. total phenolics content respectively, both of them had higher total phenolics content than other pigmented and non-pigmented maize. The average value of the total phenolics in red-yellow, red, dark red genotypes was 6056.9 mg GAE/kg

d.m. The lowest content of total phenolics was 4494.1 ± 293.8 mg GAE/ kg d.m., in multi colored maize.

Lopez-Martinez *et al.* (2009) analyzed total phenolics including free and bound phenolics, anthocyanin, and ferulic acids in eighteen strains of Mexican maize. They analyzed 4 purple maize, 4 black maize, and 6 red maize along with blue, orange, yellow and white maize; collected from local markets of different cities of Puebla, Oaxaca, Veracruz and Mexico City. Free phenolic compounds were extracted using ethanol water mixture (80:20, v/v) in a chilled waring blender. The supernatant was evaporated and reconstituted by water for analysis. Bound phenolic compounds were extracted by ethyl acetate from the neutralized residue which was treated with alkali. Total phenolics content in 18 Mexican maize phenotypes ranged from 170-3400 mg/100g of whole grain flour. The highest phenolic level (3400 ± 10.9 mg/100 g) was found in the 'AREQ516540TL' among purple phenotype group. Among red and black group of phenotypes Pinto and Mm04c1 had shown the greatest phenolic levels respectively. While white maize contained the lowest phenolics (170 ± 1.1 mg/100 g) among analyzed samples.

Lopez-Martinez *et al.* (2009) reported free phenolics in the pigmented and non-pigmented maize ranged from 33-680 mg/100 g of grain flour. Among purple, red and black group of phenotypes, AREQ516540TL (680-83 mg/100 g of flour), Pinto (50-123 mg/100g of flour) and No04C2 (101-108 mg/100 g of flour) had the highest levels of free phenolics respectively. This study showed that the percentage of free phenolics was 18-23 and in grain most phenolics were in bound form.

Lopez-Martinez *et al.* (2009) reported that bound phenolic levels were ranged from 136-2720 mg/100g whole grain flour. Purple phenotypes ranged from 381-2720

mg/100g of whole grain flour and ‘AREQ516540TL’ had the highest content among four. In red and black group of maize phenotypes, Pinto and NO04C2 contained the highest bound phenolics among the group and the range was from 354-463 mg/100 g and 232-494 mg/100g of whole grain flour respectively.

Adom *et al.* (2002) reported that Corn had the highest total phenolic content among other grains includes wheat, oats and rice. Among samples whole grain sunlite yellow corn was collected from general mill of Golen Valley, MN.

Adom *et al.* (2002) documented that the yellow corn contained 2.12 ± 0.09 µmol/ g of grain, 13.43 ± 0.59 µmol/ g of grain, and 15.55 ± 0.60 µmol/ g of grain of free phenolic, bound phenolic and total phenolic respectively.

Liu *et al.,* (2007) studied phytochemical profiles of five types of corn which were processed for masa, tortillas and tortilla chips. White, yellow, high- carotenoid, blue, and red corns were used for the study. Whole grain sample was collected from Texas Agriculture Experiment Station. Liu *et al.,* (2007) reported that differences in total phenolic contents among white, yellow, red, blue and high- carotenoid raw corns. In raw corn, TPC was ranged from 243.8 ± 4.6 to 320.1 ± 7.6 mg/100 g of dry weight sample. The highest phenolics content was found in high- carotenoid raw corn while the red ones had the lowest phenolics content. In corn most of the phenolics were in bound form. Content of bound phenolics ranged from 206 to 270 mg/100 g of dry weight. The free phenolics content in corn ranged from 35 to 50 mg/100 g of dry weight.

Hu *et al.* (2011) studied three types of waxy corn – white, yellow and black corn for their phytochemical profiles and antioxidant activities during different maturation stages. In China, waxy corns at different maturity stages are consumed as fresh or whole grain food. Types of waxy corn selected in this study, were white kernel type

‘Jinxiannuo 6’(JXN6); yellow kernel type ‘Xiannuo 301 (XN301); black kernel type ‘Jinxiannuo 8’; and yellow- kernel normal corn ‘Xinhuangdan 85’ (XHD85) which was used as control in the study. All types of corn were grown and nursed at Research Bases for Corns in China Xinzhou.

Hu *et al.* (2011) analyzed corn varieties which had different TAC at different maturation stage. At the maturity stage TAC range was from 0.09 to 276.11 mg of CGE/ 100 g of DW. Black corn contained the highest TAC (276.11 mg of CGE/100 g of DW) among other corns.

Hu *et al.* (2011) reported total content of phenolics in corns in different maturation stages. TPC range was from 0.23 to 3.88 mg of GAE/ g of DW at maturity stage. In each maturity stage, TPC level in black corn (JXN8) was much higher than other corns. Overall, based on phytochemical profile and antioxidant activity at different maturity stages of corn, it was recommended to select the best types of corn either for fresh consumption or whole grain food.

Zilic, *et al.* (2012) reported that total flavonoid content in multicolored maize was the lowest (198.99 ± 13.03 mg CE/ kg d.m.) followed by white maize (248.64 ± 3.92 mg CE/kg d.m.). Red and dark red maize contained 267.58 ± 3.49 mg CE/ kg d.m and 270.54 ± 3.26 mg CE/kg d.m. respectively. Light and dark blue maize contained 337.51± 13.04 and 307.42 ± 17.42 mg CE/kg d.m. respectively. It was also observed that, lemon yellow, yellow and orange maize had higher flavonoid contents than red and dark red maize in the report of Zilic, *et al.* (2012)

Adom *et al.* (2002) reported that free flavonoid content in corn was 0.16 ± 0.004 µmol/ g of grain. For bound flavonoid, corn had 1.52 ± 0.17 µmol/ g of grain. Overall, corn had the total flavonoid content of 1.68 ± 0.17 µmol/ g of grain.

Abdel-Aal *et al.* (2006) reported anthocyanin composition in various pigmented and non-pigmented edible and ornamental cereal grains including wheat, barley, corn, rice and wild rice. Among corn, shaman blue corn, cutie blue corn, purple corn, sweet scarlet red corn, cutie pink corn, ruby red corn, crimson red corn, and fiesta Indian multicolored corn were used for analysis. Corn samples were purchased or obtained from private grain producers and/or the retail market. Total anthocyanin content in eight pigmented corn grains showed wide variation. Total Anthocyanin Content ranged from 51µg/g - 1277 µg/g. Purple corn showed the greatest amount of TAC followed by scarlet red corn and shaman blue corn. Anthocyanins in corns were analyzed by LC-MS and results reveled that Cyanidin 3-glucoside was the most common anthocyanin in colored corns. In pink corn major anthocyanin was identified as pelargonidin 3-glucoside. Anthocyanin rich pigmented corns used in making tortillas. They might be used as functional food ingredients due to presence of high level of anthocyanin.

Liu *et al.*, (2007) reported anthocyanin content in among corn. Blue corn contained the highest amount anthocyanin content (36.9 mg of cyanidin-3-glucoside equivalent/100 g of dry weight basis), followed by red corn. Yellow corns had the lowest amount of anthocyanin (0.57 mf of cyanidin-3-glucoside equivalent/100 g of dry weight basis). They observed that in blue and red corn high amount of anthocyanin was lost due to different types of processing. Over all the best phytochemical profile was observed in the pigmented corn. Authors have been recommended the use of pigmented corn in different products in industry instead of white maize for the presence of high phytonutrients and antioxidative properties in pigmented maize.

Moreno *et al.*, (2005) studied on anthocyanin content and characterization in four native maize varieties of Mexico for their use as natural dyes. Maize varieties used in this

study were Arrocillo, Cónico, Peruano and Purepecha samples which selected on the basis of their intense purplish-red kernels with pigment in pericarp and aleurone layer.

Moreno *et al.,* (2005) reported that degermed kernel Arrocillo was the darkest maize with the highest level which had the highest amount of total anthocyanin content (TAC) (115.05 mg of anthocyanins/100 mg of sample). Pericarp of Arrocillo contained 1473 mg of anthocyanin/ 100 g sample and endosperm had 26.8 mg of anthocyanin/ 100 g sample. Degermed kernel of Cónico had only 54.0 mg anthocyanins/100 mg of sample which was the lowest TAC than other samples.

Moreno *et al.,* (2005) reported that Peruano and Arrocillo had the greatest concentration of anthocyanins in pericarp layer. On the other hand, low concentration of anthocyanins was found in the endosperm in all the four samples specially in Peruano. The maize with high anthocyanin in pericarp was recommended for beneficial source as natural dyes by the researcher.

Zilic, *et al.* (2012) reported that anthocyanins were not detected in white, lemon yellow, yellow and orange maize. The lowest content of anthocyanins was reported in red yellow maize (2.50 ± 0.06 mg CGE/kg d.m.) followed by red maize (15.43 ± 1.64 mg CGE/kg d.m.). The highest anthocyanins content was reported in dark red maize (696.07 ± 2.73 mg CGE/kg d.m.) followed by dark blue, light blue and multicolored maize respectively. They have reported that amounts of anthocyanins were responsible for the pericarp color. They assumed that the intensity of the pericarp color of white, lemon yellow, yellow and orange genotypes might be due to presence of carotenoids, flavonols and flavones. The presence of flavonols and flavones might increase the content of total flavonoids.

Lopez-Martinez *et al.* (2009) reported anthocyanin content in 18 maize phenotypes, which ranged from 1.54- 860 mg cyanidin 3-glucoside equivalents/ 100 g of whole grain flour. White maize had the lowest (1.54 ± 0.9 mg Cyanidin 3-glucoside/100 g sample) level of anthocyanin followed by orange phenotypes (30.6 ± 0.9 mg Cyanidin 3-glucoside /100 g sample). In pigmented phenotypes, purple, black and red maize had 93-851 76-120, 85-154 mg Cyanidin 3-glucoside/100 g of sample anthocyanin respectively. Among purple, red and black phenotypes AREQ516540TL, Pinto, Mm04c1 had shown the greatest levels of anthocyanins. This study also found that the phenotypes which were most abundant in phenolics also showed the abundance in anthocyanins.

Lopez-Martinez *et al.* (2009) analyzed percentage contribution of free and bound phenolics to total phenolics, percentage contribution of flavonoids to bound phenolics in eighteen strains of Mexican maize Lopez- Martinez *et al*., (2009) reported that the free phenolics were 18-23%, and 77-82% was in bound form.

Adom *et al.* (2002) reported that percentage contribution of free and bound phenolics to total phenolics, percentage contribution of flavonoids to bound phenolics in corn, wheat, oats and rice. In corn was percentage contribution for bound phenolics was 85% and 15% was for free phenolics. The bound flavonoids contribution to the insoluble phenolics was 91%.

From the review of literatures, it has been observed that in the study various types of maize including different pericarp color, size and varieties which had grown in different agronomic conditions and climate had wide diversity in phytonutrient content. Also, there was variation in extraction procedures of phytochemicals. Different extraction

solvents with various ratio, temperature was employed during extraction. All theses factors might cause nutrients variations in contents of all phytonutrients in maize.

CHAPTER III

MATERIALS AND METHODS

3.1. Sample collection and sample description

Six different pigmented maize samples were used in the present study. Samples were collected from various locations/ institutes of Bangladesh. Among the samples, the white maize, SAU Red maize and SAU purple maize were collected from Department of Genetics and Plant Breeding, Sher-E-Bangla Agricultural University. The pericarp color of the white maize, SAU Red maize and SAU purple maize were identified as white, red and purple respectively. The endosperm color of the kernels was white. The BARI Hybrid Maize-9 (BHM-9) had yellow pericarp and endosperm, collected from Bangladesh Agricultural Research Institute (BARI). Among the samples, Deep Red maize and Multi colored maize were indigenous varieties, collected from Chottogram division. Pericarp color of deep red maize kernel was identified as deep red to black. The pericarp color of multi colored maize kernel varied from yellow to deep red. Endosperms of the both samples were white. maize samples are shown in Figure 4.

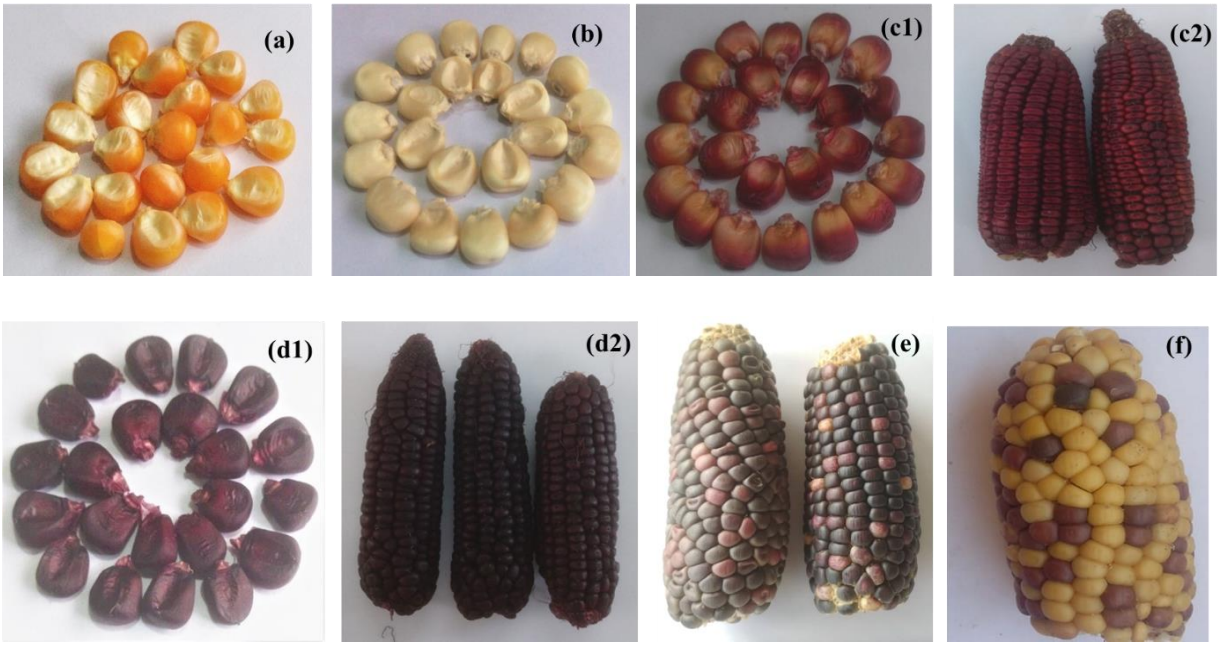

Figure 4. Experimental samples: Yellow maize (BHM-9) (a); white maize (b); SAU red maize (kernel c1, cob c2); SAU purple maize (kernel d1, cob d2); deep red maize (e); and mixed maize (f).

3.2. Sample preparation and storage

Sundried and cleaned maize kernels were pulverized to flour with a grinder (Miyako, model no: YT-4677A-S). Maize flours were stored in airtight condition and refrigerated at −20 ºC.

3.2. Apparatus

1. Beaker
2. Centrifuge (800 Centrifuge, TIN- 148-105-0491, China)
3. Centrifuge tube (15 mL & 50 mL, Corning Incorporated, Mexico)
4. Cuvette (2mm)
5. Eppendorf tubes (2 mL & 5 mL, Beijing Lab Technology, China)
6. Microcentrifuge (VS-15000N Brushless D.C motor centrifuge, Vision, Korea)
7. Micropipette (10µL – 100µL, 100µL - 1000µL, 1000µL - 5000µL)
8. Oven (Model: ISUZU SEISAKUSHO, Japan)
9. Parafilm (Bemis, USA)
10. pH meter (HI2210, Hanna instruments, Romania)
11. Reagent bottle (100 mL, 500 mL)
12. Rotter machine (FAB-LAB, SAU)
13. Shaking incubator (Model no: BJPX-103B, Biobase, China)
14. Spectrophotometer (Dynamica; HALO DB-20S, UV-VIS double beam, UK)
15. Volumetric flask (50 mL, 100 mL)
16. Vortex (Model no: VM-2000, Digisystem, Taiwan)
17. Weighing balance (New FGH Series, A&D company, Korea)

3.3. Chemicals

1. Aluminum chloride (hexahydrate) ($AlCl_3.6H_2O$) (M. W= 241.43 g/ mol, Lab-Fine, India)
2. Catechin (M.W 290.27 g/ mol, Sigma, India)
3. Citric acid (M. W=210.14 g/ mol; Merck, Germany)
4. Ferulic acid (M. W= 194.18 g/ mol, FUJIFILM WAKO, JAPAN)
5. Folin & Ciocalteu's Phenol reagent (FCR) (M. W=1.2 g/ mol; Lab Fine, India)
6. HPLC grade methanol (M. W= 32.4 g/ mol, Merck, Germany)
7. Hydrochloric Acid (HCl) (37%, M. W= 1.19 kg/ L, Merck, Germany)
8. Petroleum Benzene (Merck, Germany)
9. Potassium Chloride (KCL) (M.W = 74.56 g/ mol; Merck, Germany)
10. Sodium Carbonate (Na_2CO_3) (M.W = 1.19 kg/ L, Merck, Germany)
11. Sodium citrate (294.10 g/ mol; Lab Fine, India)
12. Sodium hydroxide (NaOH) (M. W= 40.00 g/ mol, Merck, India)
13. Sodium Nitrate ($NaNO_2$) (M. W= 69.00 g/ mol, Merck, India)

3.4. Reagent preparation

a) Preparation of acidic methanol**:** A volume of 49.5 mL HPLC methanol was taken in a 50 mL tube. Then, 0.5 mL (500µl) of concentrated HCl was added into it to obtain total volume 50 mL of acidic methanol (1%) solution.

b) Sodium carbonate Na_2CO_3 (20%) solution: In a reagent bottle, 20 g of Na_2CO_3 was taken and 100 mL of distilled water was added in it to. Total content was mixed properly to dissolved sodium carbonate properly.

c) Folin-Ciocalteu reagent (FC) reagent (1:2): A volume of 2 mL FCR was taken in a 15 mL centrifuge tube and 4 mL of distilled water was added in it and mixed properly. This reagent was freshly prepared before each experiment.

d) Sodium Nitrate ($NaNO_2$) solution: Five hundred mg of $NaNO_2$ was dissolved properly in 10 mL of distilled water in a 15 mL centrifuge tube to prepare a 1:20 ratio solution.

e) Aluminum chloride ($AlCl_3.6H_2O$) solution: In a 15 mL centrifuge tube, 1 g of $AlCl_3.6H_2O$ was added in 10 mL of distilled water. It was mixed properly to prepare a solution (1:10).

f) Sodium hydroxide (NaOH) solution (1N): To prepare 1N sodium hydroxide solution, 4 g of NaOH was taken in a 100 mL reagent bottle and 100 mL of distilled water was added in it. The solution was mixed properly to dissolved the NaOH completely.

g) Preparation of buffer solutions

- Preparation of KCl-HCl buffer (pH 1): KCl-HCl buffer was prepared using 0.2 M KCl solution and 0.2 M HCl solution as mentioned bellow. Finally, 25 mL of solution A (KCl 0.2 M) and 48.5 mL of solution B (HCl 0.2 M) were mixed together and final volume was made up to 100 mL using distilled water.

    i) Potassium chloride buffer KCl (0.2 M): In a 100 mL reagent bottle, 0.7455 g of KCl was dissolved in 50 mL of distilled water to prepare 0.2 M potassium chloride solution.

ii) Hydrochloride acid buffer HCl (0.2 M): A volume of 0.828 mL of concentrated HCl was taken in a volumetric flask and final volume was made up to 50 mL using distilled water.

- Preparation of acetate buffer (pH 4.6): Acetate buffer was prepared using 25.5 mL of 0.1 M citric acid solution and 24.5 mL of 0.1 M sodium citrate solution as mentioned bellow. The total volume was made up to 100 mL using distilled water.

i) Citric acid Buffer (0.1 M): In a 100 mL reagent bottle, 4.202 g of citric acid was added in 50 mL of distilled water. Solution was mixed well to dissolved the citric acid properly.

ii) Sodium citrate buffer (0.1 M): In a 100 mL reagent bottle, 5.882 g of sodium citrate was added in 50 mL of distilled water. Solution was mixed well to dissolved the sodium citrate properly.

h) Preparation of standards

- Preparation of ferulic acid standard: An amount of 100 mg of ferulic acid was dissolved in 15 mL of methanol (HPLC grade) in a 100 mL volumetric flask. Final volume was made up to 100 mL with distilled water. This volume was considered as stock amount. Then, 4 mL of stock solution was transferred in a 50 mL volumetric flask and final volume was made up to 50 mL with distilled water.

- Preparation of catechin standard

i) Preparation of stock (100 mg/ 25 mL) standard: In a 25 mL volumetric flask, 12.5 mL of methanol was added to 100 mg of catechin. Then total volume was made up to 25 mL by adding 12.5 mL of distilled water.

ii) Preparation of working standard: From the stock solution, 1.25mL solution was taken and the total volume was made up to 25 mL using distilled water.

3.5. Extraction of polyphenols

A) Extraction with Methanol

To prepare the Methanolic extract of samples, one gram of each sample was taken in 15 mL centrifuge tubes. Four mL of HPLC grade methanol was added to each tube. Then sample was mixed properly using a vortex mixture. Later the tubes with mixture were set to a mixture machine to mix properly for 1 hour at 2500 rpm. After 1 hour, the mixture machine was stopped and tubes were kept aside for 5 minutes. Then the tubes were centrifuged for 5 minutes at 2500 rpm. After centrifugation, the supernatant was transferred to an Eppendorf tube. The Eppendorf tube was tightly capped and wrapped with parafilm. The methanolic extracts of samples were labeled properly and preserved at 4 $^{\circ}$C. Extraction of samples with methanol is presented in Figure 5.

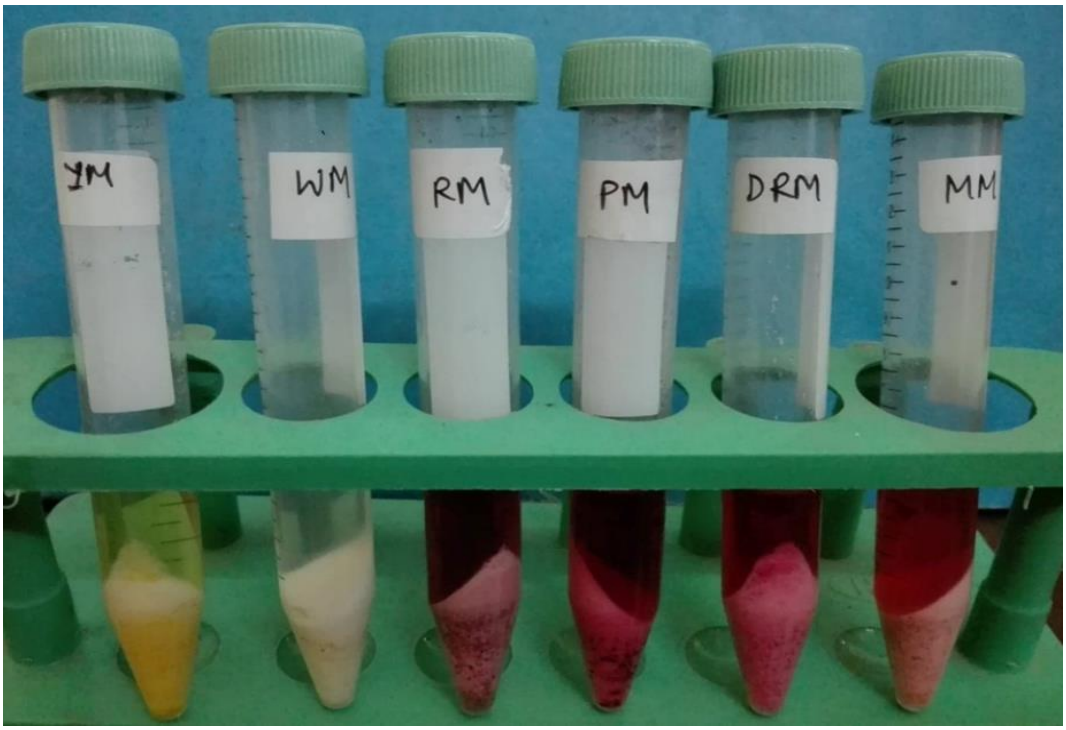

Figure 5. Extraction of polyphenols from maize samples with methanol

B) Extraction with acidic methanol

After transferring the methanol extracts, the pellets of the samples were remained in the centrifuge tubes. Then 4 mL of acidic methanol solvent was added to the pellets in each tube. Pellets and solvents were mixed well by vortex mixture. Afterwards mixing of samples with solvent and collection of supernatants from centrifuge tubes were carried out following the steps mentioned in the section 9 (A). The samples were labeled properly and preserved at 4 $^{\circ}$C. Extraction of samples with acidic methanol is presented in Figure 6.

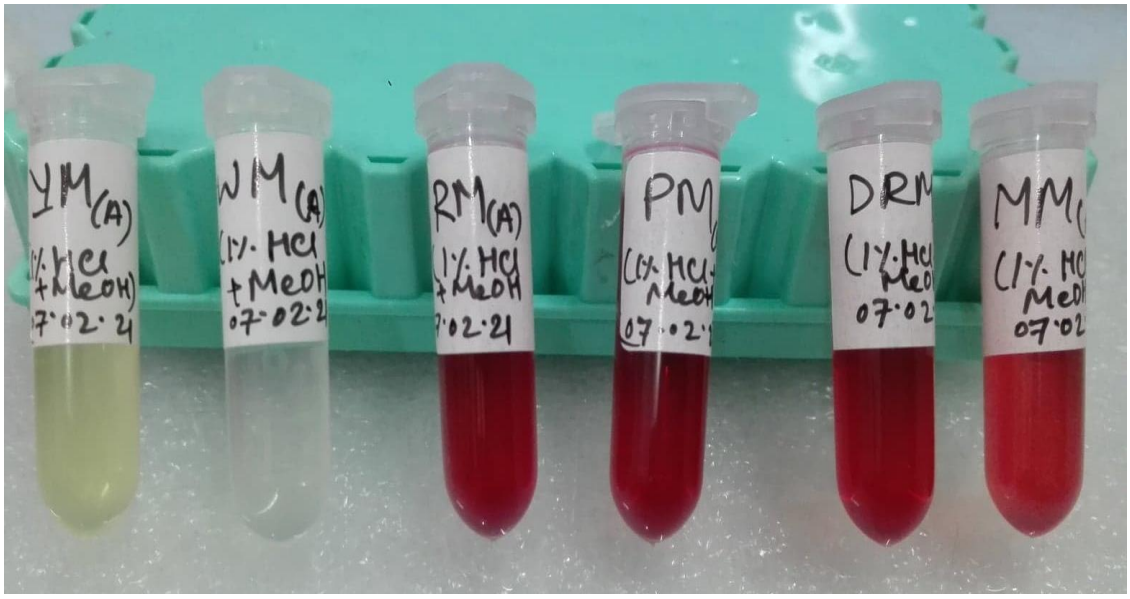

Figure 6. Extraction of polyphenols from maize samples with acidic methanol

3.6. Extraction of flavonoids

A) Defatting of samples with Petroleum Ether (b.p 60 80 ˚C)

In a 50 mL centrifuge tube, one gram of maize flour was taken and 35 mL of petroleum ether was added with it. Total content was mixed for 1 hour. Then total content was centrifuged at 3000 rpm for 5 min. After that petroleum ether was drained out without losing any sample. Again 35 mL of petroleum ether was added to the residue and the process was repeated. Finally, the sample was dried in an oven (Model: ISUZU SEISAKUSHO) for 30 minutes.

B) Preparation of acidic Methanolic extracts from the defatted samples for flavonoid determination

To prepare extracts, four mL of acidic Methanol solvent was added to dried defatted maize sample. Total content was mixed well by a vortex mixture. Later the mixture was mixed for 1 hour, after that centrifuged at 3500 rpm for 7 min at room temperature. The supernatant was collected in Eppendorf through filtering using a 0.45µm membrane filter. Extracts were stored at −4 ˚C until analyzed. These extracts were used for total flavonoids estimation. Extraction of samples with acidic methanol is presented in Figure 7 (a & b).

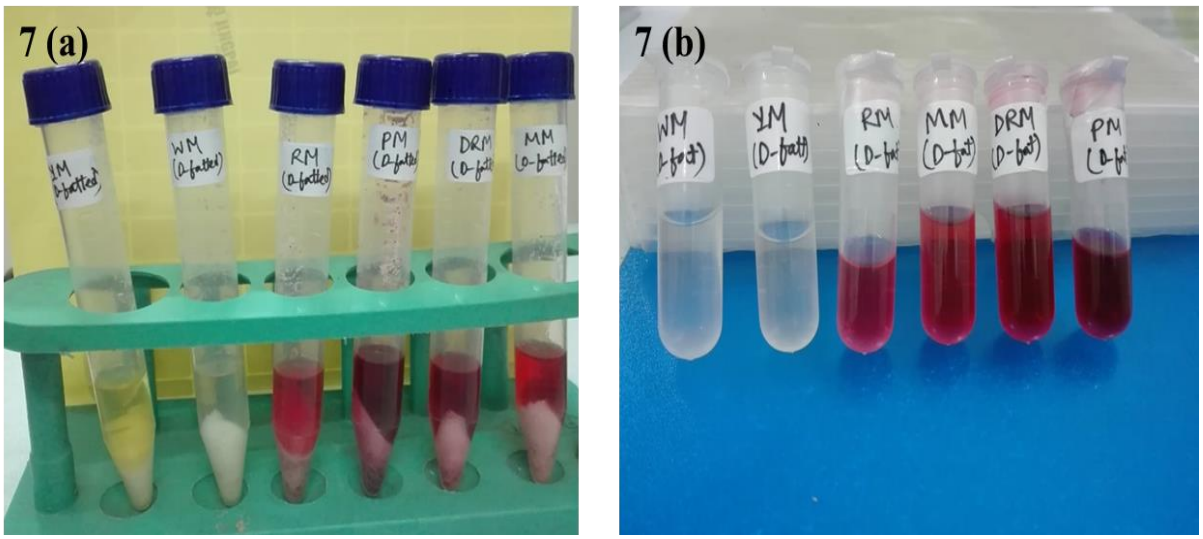

Figure 7 (a & b). Extraction of flavonoids from maize samples with acidic methanol

3.7. Preparation of extracts with acidic methanol for anthocyanin determination

To prepare the extract one gram of maize flour and 4 mL of acidic methanol were taken in a 15 mL centrifuge tube. It was mixed well by a vortex and later allowed to mix for 1 hour in mixture machine. After 1 hour, tube was centrifuged at 2500 rpm for 10 min at room

temperature. The supernatant was collected in Eppendorf through filtering by 0.45 µm membrane filter. Finally, the supernatant was stored in 2 mL Eppendorf at −4 °C.

3.9. Determination of methanol soluble polyphenol

Methanol soluble polyphenol in maize was determined by Folin-Ciocalteu reagent earlier described by Singleton, Orthofer, & Lamuela-Raventós, (1999). Briefly, in a 15 mL centrifuge tube, methanolic extract (in different volume) was taken. Final volume of extract was adjusted to 250 µL with distilled water. Then, 250 µL Folin-Ciocalteu reagent was added with it. Later 500 µL of 20% Na_2CO_3 was added in it and shaken well. Final volume was made up to 5 mL with distilled water. Total content was mixed well by vortex for few seconds. The mixture was kept in dark for 30 min undisturbed. After 30 min, the mixture was centrifuged at 2500 rpm for 5 min. In the similar way, a blank was prepared for the study. Then, the absorbance of the solution was taken at 760 nm by using spectrophotometer. Finally, soluble polyphenol in maize sample was calculated from the standard curve of ferulic acid. Polyphenol concentration was determined by using ferulic acid equivalent scale (1 OD equivalent to 0.0227 µg of ferulic acid). Soluble polyphenol of the maize sample was expressed as mg of ferulic acid equivalent/ 100 g sample dry weight.

3.10. Determination of acidic methanol soluble polyphenol

Acidic methanol soluble polyphenol in maize samples was determined from acidic methanol extracts of maize following the procedure described in the section 10. Acidic methanol soluble polyphenol was expressed as mg of ferulic acid equivalent/100 g sample dry weight.

Total polyphenol was calculated as sum of methanol soluble and acidic methanol soluble polyphenol and expressed as mg of ferulic acid/100 g sample dry weight.

3.11. Preparation of standard curve (linearity curve) of ferulic acid

The standard curve of ferulic acid was prepared from the straight-line equation, y= mx + c. Linear regression was confirmed from various concentration (3.2, 4, 4.8, ……. 8, 8.8) of ferulic acid plotted on X axis and OD obtained from those concentration on Y axis. The standard curve is shown in Figure 8.

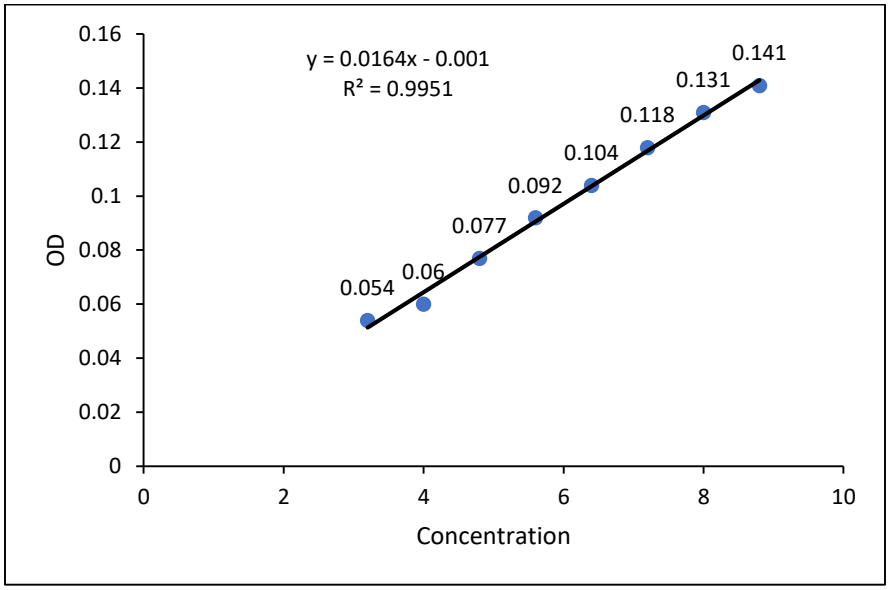

Figure 8. Standard curve of ferulic acid

3.12. Estimation of total flavonoid in maize by $AlCl_3$ method

Total flavonoids content in maize was estimated following the method by Hossain and Jayadeep, (2021). Briefly, different amount of acidic methanolic extract was taken in 1.5

mL Eppendorf. Then the volume was made up to 500 µL by using distilled water. Later 75µL of $NaNO_2$ was added in it. Then, 150 µL of $AlCl_3.6H_2O$ was added and mixed well and kept aside for 6 min. After six minutes 0.5 mL of NaOH (1N) was added in it and vortex the mixture. Total content was kept in dark for 15 min. After incubation it was centrifuged at 2500 rpm for 5 min and then kept in dark for another 15 min. Then, the absorbance was taken at 512 nm against the reagent blank. Reagent blank was used for autozero. Here, acidic methanol was used as blank. Total flavonoids in maize were calculated from the standard curve of catechin and expressed as mg of catechin equivalent/100 g sample dry weight.

3.13. Preparation of standard curve of catechin

The standard curve of catechin was prepared from the straight line equation, y=mx + c. Linear regression was confirmed from various concentration (4, 6, …..12, 14) of catechin plotted on X axis and OD obtained from those concentration on Y axis. The standard curve is shown in Figure 9.

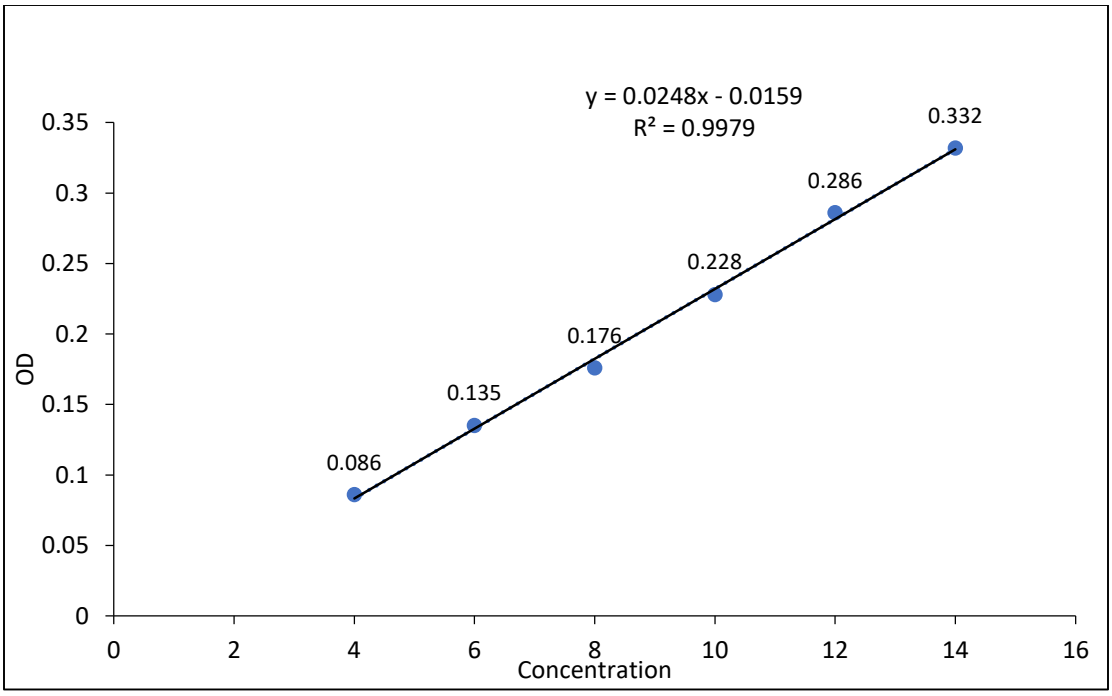

Figure 9. Standard curve of catechin

3.14. Estimation of Anthocyanin

Anthocyanin content in maize was determined following the method described by Urias-Lugo et al. (2015). To estimate anthocyanin content, different volume of acidic methanolic extract of maize was taken in 2 mL eppendorf and the total volume was made up to 1.5 mL adding buffers (pH 1 and pH 4.6). The content was mixed properly and kept for 20 minutes. After 20 minutes incubation, tubes were centrifuged and the absorption was taken at two different wave length 515 nm and 700 nm. Difference between the absorbance of two different pH was the observation. Total anthocyanin content was expressed as mg cyaniding-3 glucoside (C3G)/100 g sample dry weight.

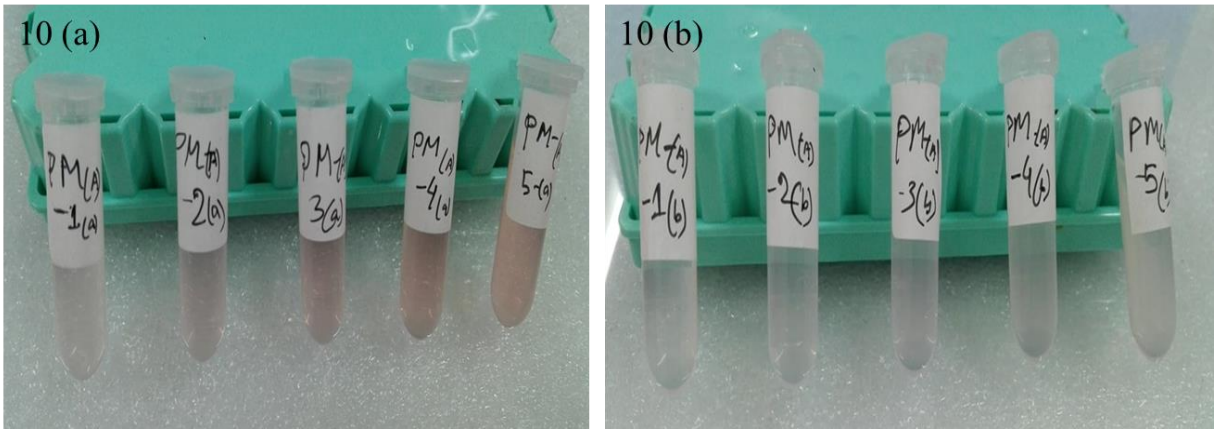

Figure 10. Extraction of anthocyanin in pH 1 buffer solution (a); and pH 4.6 buffer solution (b)

3.15. Statistical analysis

Values are presented as mean ± standard deviation (SD) of three repetitions of each experiment and calculated as dry weight basis. Means of components in samples were compared by one-way ANOVA and Tukey's test at the confidence level of 95% using IBM SPSS 20 statistical software (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

CHAPTER IV

RESULT AND DISCUSSION

4.1. Phenolic contents in maize

4.1.1 Methanol soluble phenolics

The contents of methanol soluble or free phenolics were estimated among the six pigmented and non-pigmented varieties of maize. The free phenolics contents ranged from 30.55 ± 1.61 mg FAE/100 g of flour to 105.82 ± 8.3 mg FAE/100 g of flour dry weight basis Figure 11. Among the group of samples, the SAU purple maize and the deep red maize had the highest free phenolics content which were 105.82 ± 8.3 mg FAE/100 g of flour and 105.18 ± 8.7 mg FAE/100 g of flour respectively. On the contrary, the non-pigmented white maize had the lowest amount (30.55 ± 1.61 mg FAE/100 g of flour) of free phenolics. Yellow maize, SAU red maize and mixed maize had significantly similar in free phenolics.

Lopez- Martinez *et al.* (2009) reported the free phenolics in the pigmented and non-pigmented maize ranged from 33-680 mg/100 g of grain flour. Among purple, red and black group of phenotypes were reported the highest levels of free phenolics respectively. The lower level of free phenolics found in present study was similar to the value reported by Lopez- Martinez *et al.*, (2009). However, the higher level of free phenolics reported by the authors was much higher than the present findings. Similar to their study we also observed that pigmented maize varieties content higher free phenolics compare to non-pigmented maize.

Liu *et al.* (2007) studied phytochemical profiles of five types of corn which was processed for masa, tortillas and tortilla chips. White, yellow, high- carotenoid, blue, and red corns were used for the study. In the raw corn the free phenolics content ranged from 35 to 50 mg /100 g of dry weight. Their reported value was similar to the lower range of free phenolics to our study.

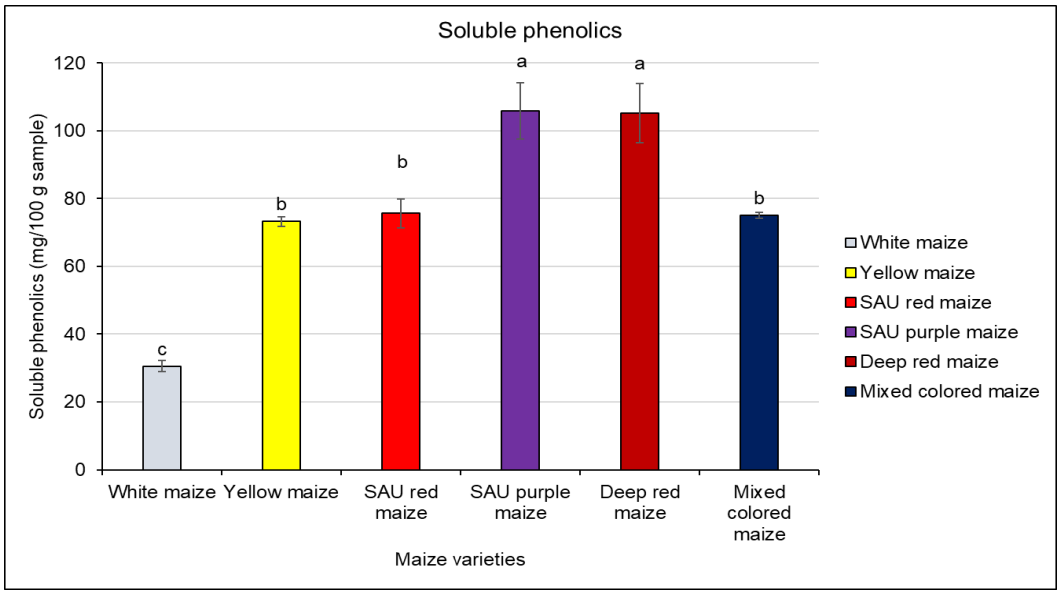

Figure 11. Methanol soluble phenolics in maize varieties

4.1.2 Acidic methanol soluble phenolics

The contents of acidic methanol soluble or bound phenolics ranged from 46.85 ± 4.4 mg FAE/100 g of flour to 165.05 ± 7.5 mg FAE/100 g of flour (Figure 12). Among the groups, the SAU purple maize and the deep red maize had the highest bound phenolics content which were 165.05 ± 7.5 mg FAE/100 g of flour and 153.11 ± 9.6 mg FAE/100 g of flour

respectively. Yellow maize (57.96 ± 4.16 mg FAE/100 g of flour), SAU red maize (55.63 ± 8.44 mg FAE/100 g of flour), and white maize (46.85 ± 4.4 mg FAE/100 g of flour) had similar bound phenolics content. Among the samples, white maize performed the lowest in bound phenolics. It has been observed that content of acidic methanol soluble phenolics was lower in yellow maize and SAU red maize than methanol soluble phenolics while it was higher in other maize samples (Figure 12).

Lopez- Martinez *et al.* (2009) reported the bound phenolic ranged from 136 to 2720 mg/100 g whole grain flour. The purple phenotypes ranged 381-2720 mg/100g of whole grain flour. In red and black group of maize phenotypes, Pinto and NO04C2 contained the highest bound phenolics among the group and the range was from 354-463 mg/100 g and 232-494 mg/100g of whole grain flour respectively. The present findings observed much lower bound phenolics than the reported value. Liu et al., (2007) also reported higher content of bound phenolics ranged from 206 to 270 mg/100 g of dry weight. They observed that the highest phenolics content was found in high- carotenoid raw corn while the red ones had the lowest phenolics content which is opposite to our observation in the present study. On the other hand, Adom *et al.* (2002) reported much lower bound phenolics content in yellow corn than the present study. Yellow corn contained 13.43 ± 0.59 µmol/ g of grain bound phenolics contents.

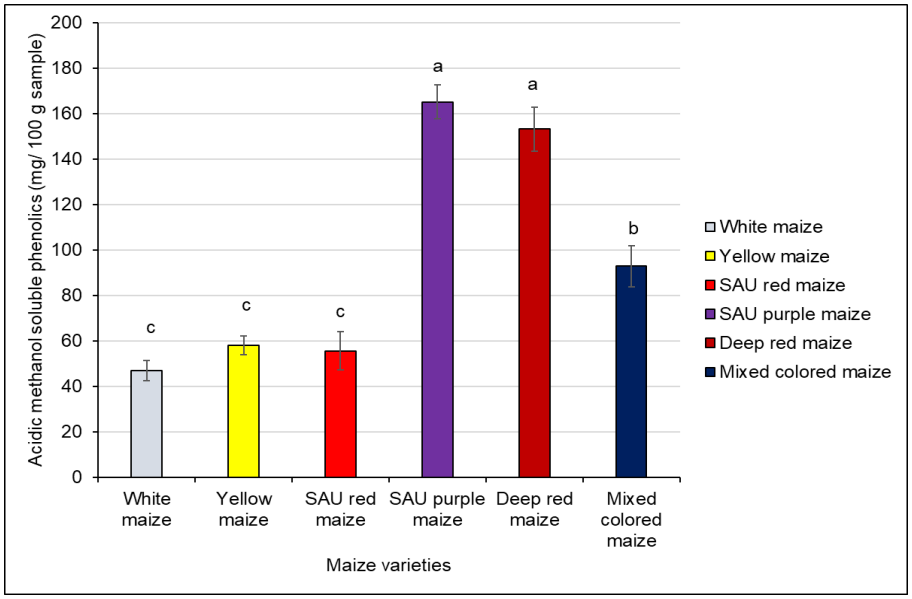

Figure 12. Acidic methanol soluble phenolics in maize varieties

4.1.3 Total phenolics

The total phenolics contains in six maize varieties were calculated from free and bound phenolics. The contents of total phenolics ranged from 77 mg FAE/100 g to 270 mg/100 g of flour (Table 2). Among the groups, the SAU purple maize and the deep red maize had the highest total phenolics content which were 270.87 ± 15.80 mg FAE/100 g of flour and 258.29 ± 5.4 mg FAE/100 g of flour respectively. There are no significant differences between yellow maize, SAU red maize and mixed maize. White maize had the lowest total phenolics content (77.41 ± 5.7 mg FAE/100 g of flour).

Zilic, *et al.* (2012) reported the total phenolics content in white, lemon yellow, yellow and orange maize ranged from 5227.1 to 5811.2 GAE/kg d. m. The light blue and dark blue genotypes had higher total phenolics content than other pigmented and non-pigmented

maize. The lowest content of total phenolics was in multi colored maize. the reported value for total phenolics in maize was much higher than the present study.

Lopez-Martinez *et al.* (2009) also reported higher total phenolics content in 18 Mexican maize phenotypes ranged from 170 to 3400 mg/100 g of whole grain flour. They reported the lowest phenolics (170 ± 1.1 mg/100 g) in white maize among analyzed samples. Liu *et al.* (2007) studied that there are significant differences in total phenolic contents among white, yellow, red, blue and high- carotenoid raw corns. In raw corn, the TPC was ranged from 243.8 ± 4.6 to 320.1 ± 7.6 mg/100 g of dry weight sample. The highest phenolics content was found in high- carotenoid raw corn while the red ones had the lowest phenolics content. In the present study, we also observed that the non-pigmented white maize contained the lowest phenolics.

Table 2. Total phenolics (mg FAE/100 g dry weight basis) in pigmented maize

Maize sample	Methanol soluble phenolics (mg FAE/ 100 g)	Acidic methanol soluble phenolics (mg FAE/ 100 g)	Total phenolics (mg FAE/100 g)
SAU purple maize	105.82 ± 8.3[a]	165.05 ± 7.5[a]	270.87 ± 15.80[a]
White maize	30.55 ± 1.61[c]	46.85 ±4.4[b]	77.41 ± 5.7[c]
Yellow maize	73.22 ± 1.39[b]	57.96 ± 4.16[b]	131.18 ± 2.96[b]
SAU red maize	75.63 ± 4.2[b]	55.63 ± 8.44[b]	131.26 ± 4.35[b]
Deep red maize	105.18 ± 8.7[a]	153.11 ± 9.6[a]	258.29 ± 5.4[a]
Mixed maize	75.01 ± 0.87[b]	92.9 ± 8.9[b]	167.92 ± 9.4[b]

All values are presented as mean ± SD (standard deviation) at dry weight basis; different alphabets in each column show the significant difference ($p < 0.05$).

4.2. Flavonoid content in maize

The contents of flavonoids were estimated among the six pigmented and non-pigmented varieties of maize. The total flavonoid contents in the maize varieties are significantly different from one another. Total flavonoids content in maize varieties ranged from 8.82 ± 1.5 mg CE/100 g to 76.49 ± 9.5 mg CE/100 g sample dry basis (Figure 13). The SAU purple maize contained the highest amounts of flavonoids (76.49 ± 9.5 mg CE/100 g sample dry basis) followed by deep red maize (52.1 ± 7.5 mg CE/100 g). Mixed maize had 18.22 ± 2.7 mg CE/100 g of flavonoids. SAU red maize, yellow maize and white maize had similar amounts of flavonoid contents (14.51 ± 1.9 mg CE/100 g, 12.4 ± 1.8 mg CE/100

g, and 8.82 ± 1.5 mg CE/100 g of sample dry basis). Among them, the white maize performed the lowest for flavonoids content. The present report was in support of the report by Zilic *et al.*, (2012). According to their report, multicolored maize and followed by white maize, red and dark red maize, and light and dark blue maize had the lowest amount total flavonoid contents. It was also observed that, lemon yellow, yellow and orange maize had higher flavonoid contents than red and dark red maize. The flavonoid content might vary according to darkness of pericarp color of maize.

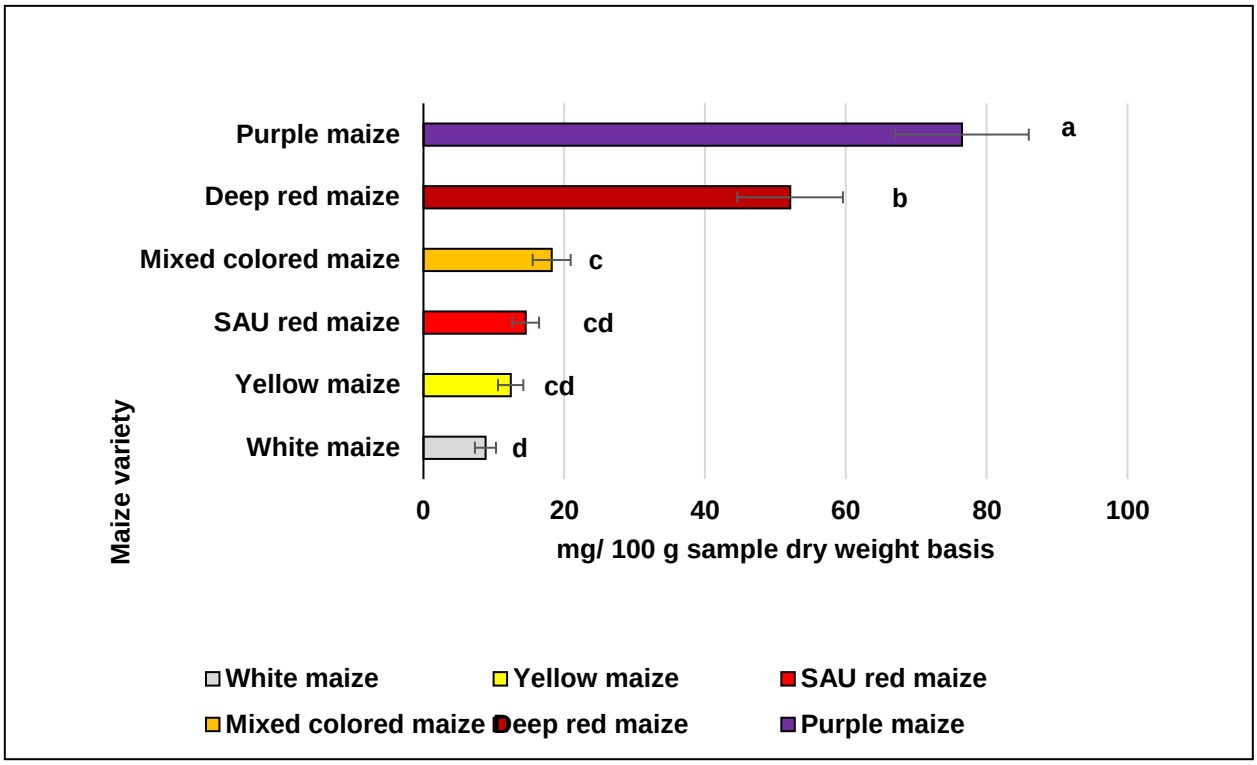

Figure 13. Total flavonoids content in maize variety

4.3. Anthocyanin in maize

The total anthocyanin contents in different varieties of maize are presented in the Figure 14. The total anthocyanin contents are significantly different from one another variety of maize and they ranged from 4.7 ± 1.1 mg to 68.58 ± 5.3 mg C3G/100 g sample dry basis.

SAU purple maize contained the highest amount of anthocyanin (68.58 ± 5.3 mg of C3G/100 g sample dry basis) among maize varieties. Deep red maize performed the next to SAU purple maize in anthocyanin content (50.14 ± 6.4 mg of C3G/100 g sample dry basis). Compared to purple maize other maize contained negligible amount of anthocyanin. Mixed maize contains a fewer amount of anthocyanin (15.1 ± 3.14 mg of C3G/100 g sample dry basis). There were no significant changes between the white, yellow and SAU red maize (5.7 ± 0.8 mg C3G/100 g sample, 4.7 ± 1.1 mg C3G/100 g sample, 4.11 ± 1.2 mg of C3G/100 g sample).

Zilic *et al.* (2012) reported that anthocyanins were not detected in white, lemon yellow, yellow and orange maize. The lowest content of anthocyanins was reported in red yellow maize followed by red maize. The highest anthocyanins content was reported in dark red maize followed by dark blue, light blue and multicolored maize respectively. They have reported that amounts of anthocyanins were responsible for the pericarp color. Similar to their study, we also observed more anthocyanins in dark colored maize and less in non-pigmented maize.

Similar to the report by Lopez-Martinez *et al.*,2009, we also observed low anthocyanin content in white maize and high anthocyanin content in pigmented phenotypes such as purple, black and red maize.

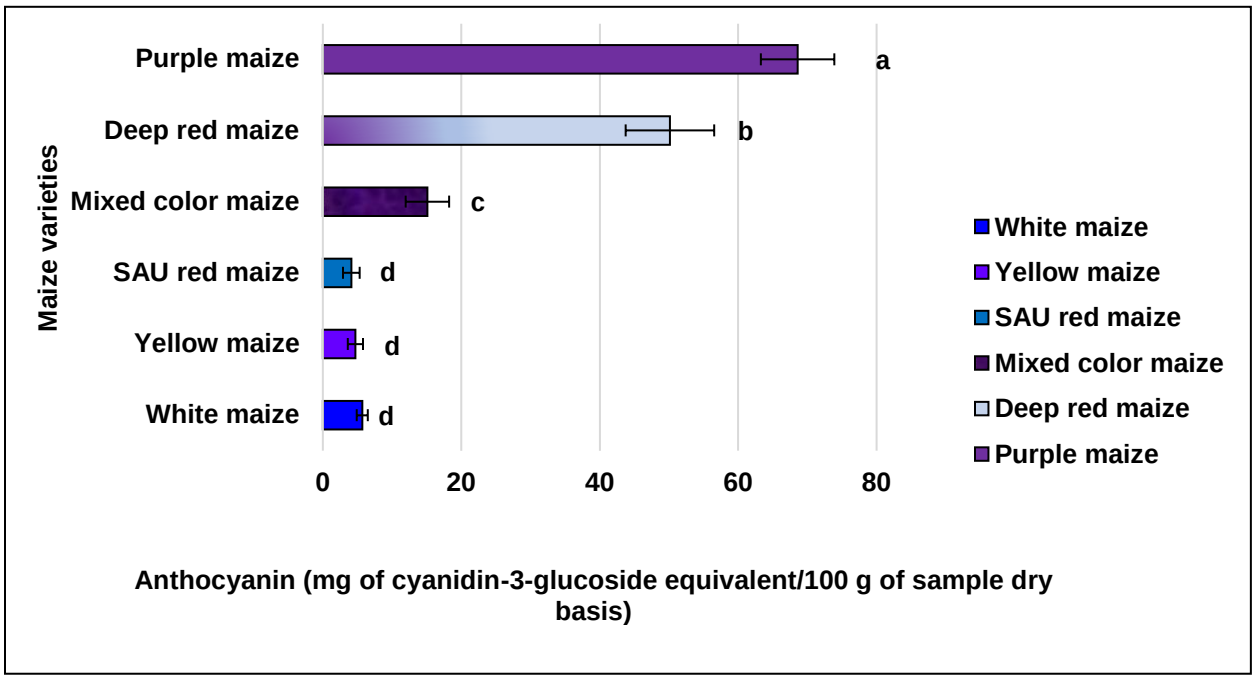

Figure 14. Anthocyanin content in maize variety

4.4. Percentage contributions of phenolics

Percentage contributions of soluble and insoluble phenolics to total phenolics, percentage contribution of flavonoids to insoluble phenolics, percentage contribution of anthocyanins to total flavonoids are presented in the Table 3. Contribution of soluble phenolics to total phenolics in maize varieties ranged from 39.07% to 55.82% while it was for insoluble phenolics to total phenolics ranged from 42.38% to 60.94%. Percentage contribution of insoluble phenolics to total phenolics was higher than its contribution of soluble phenolics except yellow maize and SAU red maize. Percentage contributions of soluble phenolics to total phenolics was the least for SAU purple maize while soluble phenolics content was

high in the SAU purple maize. Notable we observed that percentage contributions of insoluble phenolics to total phenolics in white maize was similar to its contribution of SAU purple maize. However, the insoluble phenolics content in white maize was lowest among the sample. Percentage contribution of flavonoids to insoluble phenolics ranged from 18.83% to 46.34%. The most percentage contribution of flavonoids to insoluble phenolics was observed in SAU purple maize and the least was observed in white maize. Similar observation was also for total flavonoid content in maize samples. Percentage contribution of anthocyanins to flavonoids ranged from 28.33% to 96.24%. The most percentage contribution of anthocyanin to flavonoids was observed in deep red maize and the least was observed in SAU red maize, although the highest content of anthocyanin was observed in the SAU purple maize and the lowest was observed in yellow maize.

Lopez- Martinez *et al*. (2009) reported that the free phenolics were 18-23%, while 77-82% was bound phenolics contributed to total phenolics. According to Adom et al., (2002) the bound phenolics contribution was 85% and it was 15% for free phenolics to total phenolics in corn. They also reported that bound flavonoids contribution to the insoluble phenolics was 91% in corn. Our present findings for free and bound phenolics were similar to the report. However, percentage contribution of total phenolics to bound phenolics was lower than the reported value.

Table 3. Percentage Contributions of Free and Bound Fractions of maize to Total Phenolics, total flavonoids, and total anthocyanins

Maize Sample	Phenolic content (%)		Flavonoid content (%)	Anthocyanin content (%)
	soluble	insoluble		
White maize	39.47	60.53	18.83	64.63
Yellow maize	55.82	44.18	21.39	37.90
SAU red maize	57.62	42.38	26.08	28.33
SAU purple maize	39.07	60.94	46.34	89.66
Deep red maize	40.72	59.28	34.03	96.24
Mixed colored maize	44.67	55.32	19.61	82.88

CHAPTER V

SUMMARY AND CONCLUSION

Present study was conducted to evaluate phyto-nutritional properties of maize available in Bangladesh. The study was carried out at Sher-e-Bangla Agricultural University. A wide range of hybrid and local pigmented and non-pigmented maize varieties were evaluated for the phytochemical properties such as phenolics, flavonoids and anthocyanins. SAU purple maize, SAU red maize, white maize, yellow maize, deep red maize, and mixed maize were the study materials.

Free phenolics of maize was estimated from absolute methanolic extracts of maize samples. The SAU purple maize and the deep red maize had the highest free phenolics content which were 105.82 ± 8.3 mg FAE/100 g of flour and 105.18 ± 8.7 mg FAE/100 g of flour respectively while the non-pigmented white maize had the lowest amount (30.55 ± 1.61 mg FAE/100 g of flour) of free phenolics.

Bound phenolics of maize was estimated from acidic methanolic extracts of maize samples. The SAU purple maize and the deep red maize had the highest bound phenolics content which were 165.05 ± 7.5 mg FAE/100 g of flour and 153.11 ± 9.6 mg FAE/100 g of flour respectively. White maize (46.85 ± 4.4 mg FAE/100 g of flour) performed the lowest in bound phenolics.

Total phenolics of maize was calculated from free and bound phenolics of maize samples. Among the samples, the SAU purple maize and the deep red maize had the highest total phenolics content which were 270.87 ± 15.80 mg FAE/100 g of flour and 258.29 ± 5.4 mg

FAE/100 g of flour respectively while the white maize had the lowest total phenolics content (77.41 ± 5.7 mg FAE/100 g of flour).

The SAU purple maize contained the highest amounts of flavonoids (76.49 ± 9.5 mg CE/100 g sample dry basis) and white maize (8.82 ± 1.5 mg CE/100 g of sample dry basis), performed the lowest for flavonoids content among the samples.

SAU purple maize contained the highest amount of anthocyanin (68.58 ± 5.3 mg of C3G/100 g sample dry basis) among maize varieties. SAU red maize (4.11 ± 1.2 mg of C3G/100 g sample) performed the lowest among the samples.

Contribution of soluble phenolics to total phenolics in maize varieties ranged from 39.07% to 55.82% while it was for insoluble phenolics to total phenolics ranged from 42.38% to 60.94%. Percentage contribution of flavonoids to insoluble phenolics ranged from 18.83% to 46.34%. Percentage contribution of anthocyanins to flavonoids ranged from 28.33% to 96.24%. Percentage contribution of insoluble phenolics to total phenolics, and flavonoids to insoluble phenolics was great in the SAU purple maize. Percentage contribution of soluble phenolics to total phenolics was great in SAU red maize and it was great in deep red maize for anthocyanin to total flavonoids content.

It was observed that a relationship between the enrichment of anthocyanins were related to the levels of phenolics. The most phenolics rich variety; SAU purple maize exhibit the greatest level of phenolics, flavonoid and anthocyanins. Deep red maize and mixed maize had also shown a vast of content respectively. These varieties should have further more studies for other phytonutrients contents and their potential health benefits.

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