EVALUATION AND MODIFICATION OF PROCESSING TECHNIQUES FOR RECOVERY OF ANTHOCYANINS FROM COLORED CORN

BY

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DISSERTATION

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Abstract

Color is the most important indicator of taste and flavor, both vital sensory properties of food and beverages. The processing industry commonly uses artificial food colorants derived from petroleum or coal, called coal tar dyes. These dyes may cause attention-deficit/hyperactivity disorder, genotoxicity, neurotoxicity and/or carcinogenicity. In an era of "natural ingredients", there is increasing customer pressure for exploring natural alternatives of synthetic dyes. Anthocyanins can be used as a replacement of synthetic FD&C Red 40 dye. However, their recovery from conventional sources is uneconomical due to higher inputs and lower economic value of the processing residues. The potential of using colored corn as a viable source of anthocyanins was explored.

Anthocyanins containing purple and blue corn were fractionated using laboratory scale wet milling, dry milling and dry grind processes; coproduct yields were compared with those from conventional yellow dent corn. In wet milling, starch yields of colored corn were 6.7 to 8.7% less than conventional yellow dent corn on dry basis (db). In dry milling, large grit yields of colored corn were <25% (db), implying a softer endosperm composition. In the dry grind process, mean final ethanol concentrations for colored corn were 2.7% (v/v) less than those from yellow dent corn. Colored corn may be used in all three processes; however, with some yield differences. Colored corn coproducts from three processes were analyzed for anthocyanin content. Corn processing can generate coproducts with disproportionately higher anthocyanin contents. Location of pigments in colored corn kernels differed. Anthocyanins in the blue and purple corn were located in the aleurone and pericarp, respectively. Purple corn contained 13 times higher anthocyanins compared to blue corn. For purple corn, 80% of the anthocyanins were found in steepwater from wet milling and 48% in pericarp from dry milling process. Corn

processing was tailored in such a way so as to recover coproducts with maximum anthocyanin concentrations while other fractions, which remain unaffected, could be further utilized.

To increase anthocyanin recovery, dry milled purple corn pericarp was steeped using different combinations of wet milling chemicals. In the treatment containing SO_2 (0.2%), lactic acid (0.5%) and water, 22.9 g anthocyanins/kg pericarp was quantified while the treatment containing only water recovered 7.1 g anthocyanins/kg pericarp. Although addition of SO_2 facilitated higher anthocyanin extraction, it had a bleaching effect on the final extract color. Cyanidin-3-glucoside and its acylated form were the most dominant anthocyanins in treatments containing SO_2 and lactic acid while the condensed forms were most abundant in the treatment containing only water. A 100 g dry milling protocol was developed which allowed estimation of coproduct yields for corn hybrids with low standard deviations of means. Crude oil contents of large and medium grits from hard endosperm varieties were comparable to those reported for similar lab scale studies. Due to the absence of a roller milling step, true sized large grits were recovered. This protocol should be helpful in estimating dry milling characteristics of newly developed colored corn varieties with relatively small amounts of material.

Keywords: Colored corn; anthocyanins; artificial food colorants; natural food colors; wet milling; dry milling; dry grind process

न हि ज्ञानेन सदृशं पवित्रमिह विद्यते |४-३८|

There exists nothing as purifying as knowledge|4-38|

Bhagwad Gita
500~200 BCE

Dedicated to my father and mother

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Finally, Karl Popper's quote "Our knowledge can only be finite, while our ignorance must necessarily be infinite" springs to my mind, fills me with humility and motivates to carry on from here.

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List of abbreviations

AAU	Alpha amylase units
AD	Absolute density
ADHD	Attention-deficit/hyperactivity disorder
ANC	Anthocyanins
ANOVA	Analysis of variance
BCFM	Broken corn and foreign material
C3G	Cyanidin-3-glucoside
C36MG	Cyanidin-3-O-(6”-malonyl-glucoside)
CF	Condensed form
CFR	Code of federal regulations
CP	Crude protein
DDGS	Distillers dried grain with soluble
FDA	Food and drug administration
FD&C	Food, drug, and cosmetic
GAE	Gallic acid equivalent
GAU	Glucoamylase units
GEM	Germplasm enhancement of maize
GLM	Generalized linear model
HPLC	High performance liquid chromatography
L.A.	Lactic acid
LC/MS	Liquid chromatography/mass spectrometry

LSD	Least significant difference
MA	Monomeric anthocyanins
NDF	Neutral detergent fiber
P3G	Pelargonidin-3-glucoside
P36OMG	Pelargonidin-3-O-(6”-malonyl-glucoside)
Peo3G	Peonidin-3-glucoside
Peo36OMG	Peonidin-3-O-(6”-malonyl-glucoside)
SD	Standard deviation
TKW	Thousand-kernel weight
TW	Test weight

Chapter 1. Introduction

The food processing industry mainly utilizes artificial food colorants derived from petroleum or coal, called coal tar colors, to impart vivid colors to various foods and beverages (Arnold et al. 2012). Although artificial food colorants do not contribute nutritionally, color is the single most important visual indicator of taste and flavor, both vital sensory properties of food (Delwiche 2012; Spence et al. 2010; Valentin et al. 2016). It is therefore no surprise that the use of colors in wine has been documented as early as 400 BC while colored candies can be traced back to the ancient Egyptians (Downham and Collins 2000). Historically, a number of colorful but toxic inorganic salts such as mercury sulfide, lead chromate, lead oxide, copper carbonate and copper sulfate have been used with impunity to improve food appearance (McKone 1991). However, with advances in science, toxic effects of these salts became evident and were replaced gradually ever since the first synthetic organic dye (aniline purple) was synthesized in 1856 by William Perkins from coal tar (Garfield 2002). Harvey W. Wiley (1844-1930) also known as "the father of the Pure Food and Drugs Act" did considerable work towards improving food safety in the US. Due to his efforts, seven coal tar dyes were termed safe and included for use in the Pure Food and Drugs Act, 1906 (Sharma et al. 2011).

Safety concerns arising from the ingestion of synthetic dyes are as old as the dyes themselves and have become more pronounced in recent times. Since 1960, more than 100 color additives have been banned due to safety concerns (FDA 2016). Feingold (1975) studied the effects of artificial food colors/preservatives and salicylate containing foods on the behavior of adults and children and found that diets free from artificial additives resulted in reduced restlessness, sleeplessness, inattention and aggression. Considerable research has been conducted albeit with inconclusive results to ascertain whether consumption of synthetic colors in food

cause attention-deficit/hyperactivity disorder (ADHD) in children (Bateman et al. 2004; Nigg et al. 2012; Schab and Trinh 2004; Weiss 2011). A five-fold increase in average per capita daily intake of synthetic dyes, from 12 mg in 1950 to 61 mg in 2010 has fueled consumer concerns (Arnold et al. 2012). In 2007, the Food and Drug Administration (FDA) certified 5.8 million kg of synthetic dyes for use in the US (Sharma et al. 2011). FD&C Red 40 (also called Allura Red AC) which constituted 40% of the total is of concern not only for ADHD syndrome but also for containing small amounts of benzidine which is a potential human and animal carcinogen (Potera 2010). Due to increased consumer awareness, there is an added impetus for the use of natural colors in foods which is driving industry personnel to look for economically viable sources of natural colors.

Anthocyanins, belonging to a group of water soluble pigments are phytochemicals which are responsible for red, purple and violet colors in fruits, vegetables and flowers and can be used as a natural alternate to Red 40 (Mateus and de Freitas 2009). In addition, anthocyanins also have been shown to impart several health benefits to humans as antioxidant (Cao et al. 1997), anti-diabetic (Huang et al. 2015), anti-hypertension (Shindo et al. 2007) and anti-carcinogenic compounds (Wang and Stoner 2008). Stability of anthocyanins depends upon their structural features and on a number of other factors such as solution pH, temperature, contact with oxygen, presence of ascorbic acid and light (Brouillard 1982).

Anthocyanins currently are recovered from red colored fruits and vegetables such as black grapes, blackberries, purple carrots and purple fleshed sweet potatoes using solvent extraction methods (Assous et al. 2014; Barnes et al. 2009; Bridgers et al. 2010; Dia et al. 2015; Long et al. 2013; Revilla et al. 1998). Relatively higher costs of starting material, extraction costs coupled with the fact that processing wastes after anthocyanin extraction cannot be utilized

makes overall anthocyanin recovery expensive. As a result, there is not a single commercially viable food product colored with anthocyanins currently available in the US market.

However, some colored corn cultivars contain considerable amounts of anthocyanins and can be utilized for their extraction. Due to color and chemical/biological characteristics of colored corn anthocyanins, they can be an attractive natural alternative to artificial colorants used in foods. Corn is the primary US food grain, accounting for about 95% of total food grain production and use. In 2014, 6.9 billion bushels of corn was processed by the corn industry into a variety of food ingredients and industrial products such as ethanol, starch, sweeteners, cooking oil and fuel ethanol (NCGA 2015; USDA 2015).

The main aim of this work was to explore the possibility of economic recovery of anthocyanins from colored corn cultivars and to determine whether coproducts could be utilized further, lowering overall anthocyanin recovery costs. Colored corns were fractionated using three conventional corn fractionation processes namely wet milling, dry milling and dry grind to ascertain how various milling characteristics of these corns corresponded to those of yellow dent corn. The second step was to determine the distribution of anthocyanins in various coproduct streams of these three processes with the explicit aim of identifying the coproduct containing maximum amount of anthocyanins. Once it was realized that purple corn (*Zea mays L.*) contained considerable anthocyanins concentrated in the pericarp, the next step was to optimize anthocyanin recovery from pericarp using commonly used wet milling chemicals.

During the course of this work, several new colored corn cultivars were bred at University of Illinois and there arose a need for processing small amounts of corn; as a result a 100 g dry milling protocol was developed.

Specific objectives were:

1) To compare coproduct yields of purple, blue and yellow dent corn from various milling processes (Chapter 3).
2) To study the distribution and yield of purple and blue corn anthocyanins in coproducts of wet milling, dry milling and dry grind processes (Chapter 4).
3) To increase anthocyanin recovery from purple corn pericarp using wet milling chemicals (Chapter 5).
4) Development of a 100 g corn dry milling protocol for the lab scale measurement of coproduct yield and composition (Chapter 6).

Chapter 2. Literature review

2.1. Food colors: a historical perspective

Artificial food colorants are synthetic dyes, pigments and similar substances which are used in processed foods to make them attractive, appealing and informative to the customers. They play an important part in attracting customer interest since visual inspection is often the first opportunity for customers before deciding to purchase or taste something. Color is the most important visual attribute for sensory properties of food, namely, taste and flavor. Bright natural colors are often associated with fresh product while pale colors may imply stale products with lower quality (Chylinski et al. 2015; Clydesdale 1993; Delwiche 2012; Spence 2015; Valentin et al. 2016). Slightly green bananas have a different taste as compared to yellowish-brown ones while olive-brown canned beans are not expected to taste as good as freshly harvested beans (Cardello 1995). Our expectations of relationship between food color and its taste are built on similar experiences. While purchasing food, examination and final choice is mostly based on visual inspection and associating the food with the inherent idea of how we perceive it to be. Researchers have suggested that human experience of flavor is largely dictated by our expectations (Deliza and MacFie 1996). This is the main reason appearance of food products is given higher priority than issues associated with flavor (Galaffu et al. 2015). A good example is that of tomatoes whose breeding is dictated by the need for attractive color, shape and texture of the peel to survive long distance transportation, however, this is done often at the expense of taste (Causse et al. 2010).

Therefore, it should not be surprising that smoke and aloe extract were used to improve taste and flavor of wines as early as 400 BC while early Egyptians colored their candies (Downham and Collins 2000; Sharma et al. 2011). Until 18th century, the only means available to

impart color to the food material were natural. Spices, flowers and minerals as well as animal derived pigments such as carminic acid from cochineal insects and squid ink were used to improve food appearance. In order to prevent adulteration of saffron (used to give yellow hues to the food material) with turmeric, the first European food laws enacted in 1531 at Augsburg in Germany stipulated that the guilty be burned at the stake (Stich 2016). The advent of industrialization and increasing trade between Europe and Asia introduced new food stuffs such as tea, coffee and chocolate as well as marked the beginning of food processing industry which catered to the needs of the new working class. As food processing and preservation techniques improved, the volume of processed foods multiplied and there arose a need for artificial food colorants to restore original colors of processed food. With the era of food processing came the era of industrial food colors (Downham and Collins 2000).

Due to stiff competition and in absence of regulations, all sorts of low cost metal and mineral compounds were used for disguising low quality food with disregard to safety. Colorful but toxic inorganic salts and heavy metals such as copper sulfate, lead chromate and mercury sulfide were added to improve appearance of jam, butter, pickles and candy. Copper carbonate was used to improve the color of tea while mercury sulfide and lead oxide were used to adulterate cayenne and curry powder (Hutt and Hutt 1984; McKone 1977). However, towards the end of 19th century, scientists were able to identify contaminants in food and several books and articles were published criticizing the use of food adulterants. In 1820, German chemist Friedrich Accum published pioneering work entitled “Treatise on adulterations of food and culinary poisons” criticizing the use of chemical additives in food (Brown 1925). Due to rising concerns, use of heavy metal salts in foods was banned in many countries by the first food safety legislations.

In 1856, the first synthetic organic dye called mauvine (aniline purple) was synthesized from coal tar by English chemist William Perkin. This initiated further development of coal tar based colorants for food and textile industries. Since aromatic precursor chemicals were derived from coal tar, these were called coal tar dyes (Filarowski 2010). Hundreds of coal tar colorants were soon available in the market and were increasingly used on account of wider available palette, superior stability, cost effectiveness, ease of production and no effect on food flavors (Downham and Collins 2000). Soon markets in US and Europe had many colorful, organic dyes which were used to improve appearance of ford products ranging from mustard to ketchup and jellies to wine; albeit with little regard to their toxicity or adverse health effects. At the same time, unscrupulous traders misused these dyes not only to disguise poor quality food but also to imitate more expensive ingredients. However, due to their unabated use and lack of scientific studies concerning their toxicity, safety concerns grew among the population. As a result "The Pure Food and Drugs Act" was enacted in 1906 which allowed the use of only seven coal tar dyes in foods in the US (Sharma et al. 2011).

2.2. Artificial food colorants and associated health concerns

Since the Second World War, human dependence on processed foods has increased dramatically. Today, a large proportion of food is preprocessed and ready to be prepared, keeping in mind the needs of a large working population as well as increasing number of elderly in western countries. Therefore, food industry personnel face the challenge of providing large quantities of visually appealing foods that not only taste good but also meet quality and cost criteria. As a result, per capita intake of artificial food colorants has increased steadily. The amount of synthetic dyes certified by FDA for use has increased from 1.6 million pounds in 1950 to 11.8 million pounds in 2007; this amount is estimated to have reached 17 million pounds by

2015 (CSPI 2016; Sharma et al. 2011). Although advantageous in many other aspects, some of the drawbacks associated with synthetic dyes include their decolorization in presence of ascorbic acid, precipitation due to presence of metal ions, loss of color due to microbial attack and reaction with proteins at high temperatures resulting in color fading (Downham and Collins 2000).

Use of food colors in the US is governed by the Code of Federal Regulations (CFR) which consists of 50 titles. The Food and Drug Administration has been assigned title 21; color additives are listed in parts 70-82 and divided into two main categories, namely certified color additives (FD&C colors) and colorants exempt from certification (Downham and Collins 2000). FD&C colors are synthetically produced organic molecules whose purity is verified by the FDA. Currently there are seven FDA approved food colors namely FD&C Blue 1, FD&C Blue 2, FD&C Red 3, FD&C Red 40, FD&C Yellow 5, FD&C Yellow 6 and FD&C Green 3. These are available in water soluble dyes as well as six water insoluble lakes. A lake pigment is made by precipitating a synthetic dye with an inert binder usually a metallic salt.

As the application of synthetic dyes in foods proliferated in 1960s and 1970s, Dr. Ben Feingold, a physician, strived to find out whether hyperactivity in children was related to food additives and synthetic dyes. Feingold (1975) reported that food additives such as synthetic colors, preservatives and naturally occurring food salicylates had the potential to cause behavioral disturbances in children such as ADHD. In a double-blind, placebo-controlled study involving 277 preschool children who were fed synthetic dyes and sodium benzoate (a preservative), it was found that ingestion of 20 mg of mixed dyes and 45 mg of sodium benzoate could induce hyperactive behaviors (Bateman et al.. 2004). In another study by McCann et al. (2007), 297 children ranging from three to nine years were fed two different mixtures of food

containing azo dyes and sodium benzoate. It was reported that the mixtures could provoke ADHD symptoms in children although it could not be established whether only the dyes or sodium benzoate or the combination of both caused the symptoms.

The azo dyes which are the most widely used synthetic dyes in food industry, account for 65% of the commercial dye market due to their superior stability and low cost of production (Ahlström et al. 2005). These dyes contain one or more azo bonds (R-N = N-R´) in their chemical structure. Although stable under aerobic conditions, once consumed, these dyes become reduced by intestinal flora resulting in formation of aromatic amines which may cause headaches (Hawley and Buckley 1976), genotoxicity (Mpountoukas et al. 2010), neurotoxicity (Nagaraja and Desiraju 1993) and carcinogenicity in the humans (Khehra et al. 2006). FD&C Red 40, FD&C Yellow 5 and FD&C Yellow 6 are three azo dyes permitted for use by the food industry in the US (Yamjala et al. 2016). Out of these, FD&C Red 40 alone contributes to 40% of the total synthetic dyes used by the food industry (Potera 2010).

Although studies have shown that synthetic dyes and food additives may trigger adverse reactions in some people, the mechanism of their metabolism is not fully understood and no concrete link between their intake and neurological functioning has been established. The FDA assertion that a dye will not cause a life time risk greater than one cancer in one million people, has failed to convince many skeptics. In an age when people are becoming more and more aware of what they consume and the general trend is towards “natural ingredients”, there is a pressure on the food industry personnel to explore natural alternatives for synthetic dyes. These alternatives should not only be of similar quality and stability as that of synthetic dyes but also need to be economical. All these factors have spurred a new natural color industry which is all set to grow in future.

2.3. Natural food colorants

Nature has many colors with fruits, vegetables, flowers, seeds and roots showing a wide palette. The human diet contains a variety of pigments such as anthocyanins, betalains, carotenoids, chlorophylls and curcumin. Betalains are nitrogen containing pigments which are water soluble and give red-violet (betacyanins) and yellow (betaxanthins) colors to various fruits and vegetables such as red beet, amaranth and cactus fruits. They are stable in pH from 3 to 7 and are suitable for low acid and neutral foods. Carotenoids are the most widely encountered pigments in nature and give red, orange and yellow hues to various fruits and vegetables. More than 60 carotenoids have been identified in nature and their main sources are carrots, tomatoes, red peppers, oranges and corn. Intake of carotenoids is associated with many health benefits to the humans. Chlorophyll is a green pigment found in plants and algae and plays an important role in photosynthesis. Their molecular analogs, known as chlorophyllins are more stable and used as natural colors in foods. Green leafy vegetables such as spinach, kale and lettuce are rich in chlorophyll. Curcumin is extracted from the rhizomes of *Curcuma longa* (turmeric) plant and imparts yellow color to the plant as well as to the curries made with turmeric powder in the Indian subcontinent. It has been shown to possess anti-bacterial and anti-carcinogenic properties but has low solubility in water. Some natural pigments and their important attributes are summarized in Table 2.1. Natural pigments vary greatly in their physical and chemical properties. As compared to synthetic dyes, natural pigments are expensive, less stable (on account of higher sensitivity to pH and light) and difficult to use due to their varying solubility.

TABLE 2.1. Some natural pigments and their characteristics.

Pigment	Sources	Color	Remarks
Anthocyanins	Dark fruits and vegetables	Red, pink, mauve, blue	Water soluble pigments, color is pH dependant
Betalain	Red beetroot	Pink to red	Water soluble, degraded at high temperatures
Carbon black	Vegetable material	Grey to black	Insoluble pigment, good stability
Capsanthin	Paprika	Orange to red	Oil soluble carotenoid pigment
Carminic acid	Cochineal insect	Orange to red	Water dispersible pigment. Not suitable for vegetarians
Chlorophyll	Grass, nettle	Olive green	Oil dispersible, dull color with poor stability
Crocin	Saffron flower	Yellow	Water soluble carotenoid pigment
Curcumin	Turmeric root	Bright yellow	Non soluble pigment, light sensitive
Lutein	Marigold	Golden yellow	Oil soluble pigment, only allowed for chickenfeed in the US
Lycopene	Tomato	Orange to red	Oil soluble carotenoid, poor stability

2.3.1. Anthocyanins: an introduction

Anthocyanins are water soluble vacuolar pigments which belong to the broad class of polyphenols called flavonoids (Harborne and Williams 2001). The word anthocyanin is a combination of two Greek words, “Anthos” meaning flower and “kyanos” meaning dark blue, revealing its function as a natural pigment in plant matter (Delgado-Vargas et al. 2000). While carotenoids, which are liposoluble compounds, impart yellow and red colors (Castañeda-Ovando

et al. 2009); water soluble anthocyanins, depending upon pH conditions, are responsible for imparting blue, purple, red, black and other intermediate hues to flowers, fruits, vegetables, leaves, stem, seeds and other tissues (Clifford 2000). Anthocyanins are reported to play a prominent role in plant physiology. They assist plants in pollination as well as seed dispersal, protect them from harmful UV radiation and provides them anti-viral and anti-microbial capabilities (Wrolstad 2004).

The basic building blocks of anthocyanins are anthocyanindins, also known as aglycons. They consist of an aromatic ring bonded to another heterocyclic ring containing oxygen which is further bonded to a third aromatic ring by a carbon-carbon bond, the carbon skeleton is C-6 (A ring)-C-3 (C ring)-C-6 (B ring). The glycosidic forms of anthocyanidins (bonded to a sugar moiety) are called anthocyanins (Konczak and Zhang 2004). Different colors exhibited by anthocyanin molecules are due to the resonant structure of flavylium (2-phenylchromenylium) cation, first explained by Pauling in 1939 (Wrolstad et al. 2005). There is a huge diversity of anthocyanins in nature and more than 600 anthocyanins and 25 anthocyaninidins have been identified (Anderson and Jordheim 2006). The structural differences in anthocyanins are due to the variation in number of hydroxylated groups attached, the nature and number of bonded sugars and whether aliphatic or aromatic carboxylates are bonded to the sugars and their respective positions (Kong et al. 2003). Out of 25 anthocyanidins, only six are most commonly found in the vascular plants and 95% of the anthocyanins are derived from these six. Their distribution is cyanidin-3-glucoside (50%), delphinidin-3-glucoside (12%), pelargonidin-3-glucoside (12%), peonidin-3-glucoside (12%), petunidin-3-glucoside (7%) and malvidin-3-glucoside (7%) (Castañeda-Ovando et al. 2009; Clifford 2000). Chemical structures of six main

anthocyanidins are depicted in Fig. 2.1. Non-methylated anthocyanidins have been shown to be more prevalent in nature than their methylated counterparts (Kong et al. 2003).

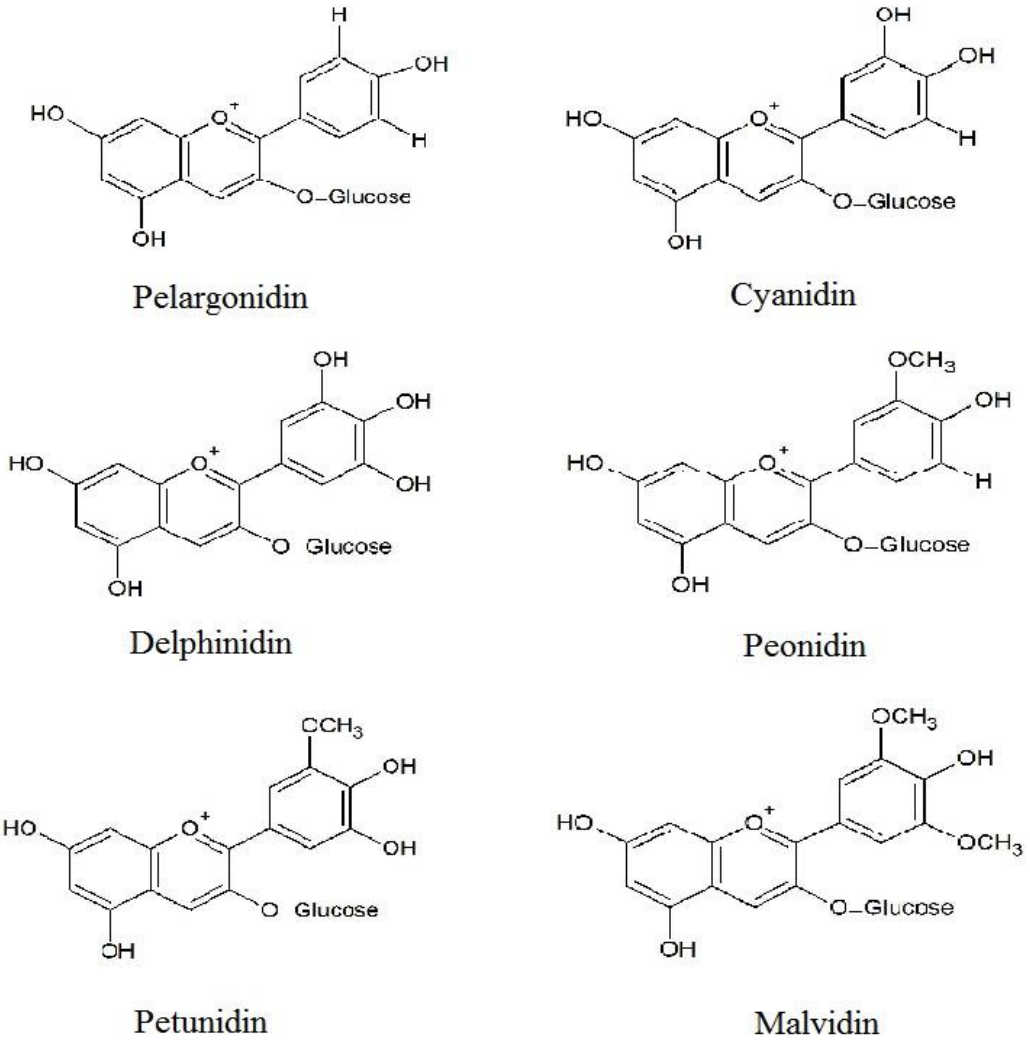

Fig. 2.1. The chemical structures of six main anthocyanidins present in nature.

Depending upon the solution pH, anthocyanins can be found in many different chemical forms displaying different hues. Flavylium cation is the most dominant species at pH 1 responsible for red and purple colors. The quinoidal blue is predominant species at pH values

between 1 and 2. Only colorless species, namely, carbinol pseudobase and chalcone are observed when pH lies between 5 and 6 (Fig. 2.2).

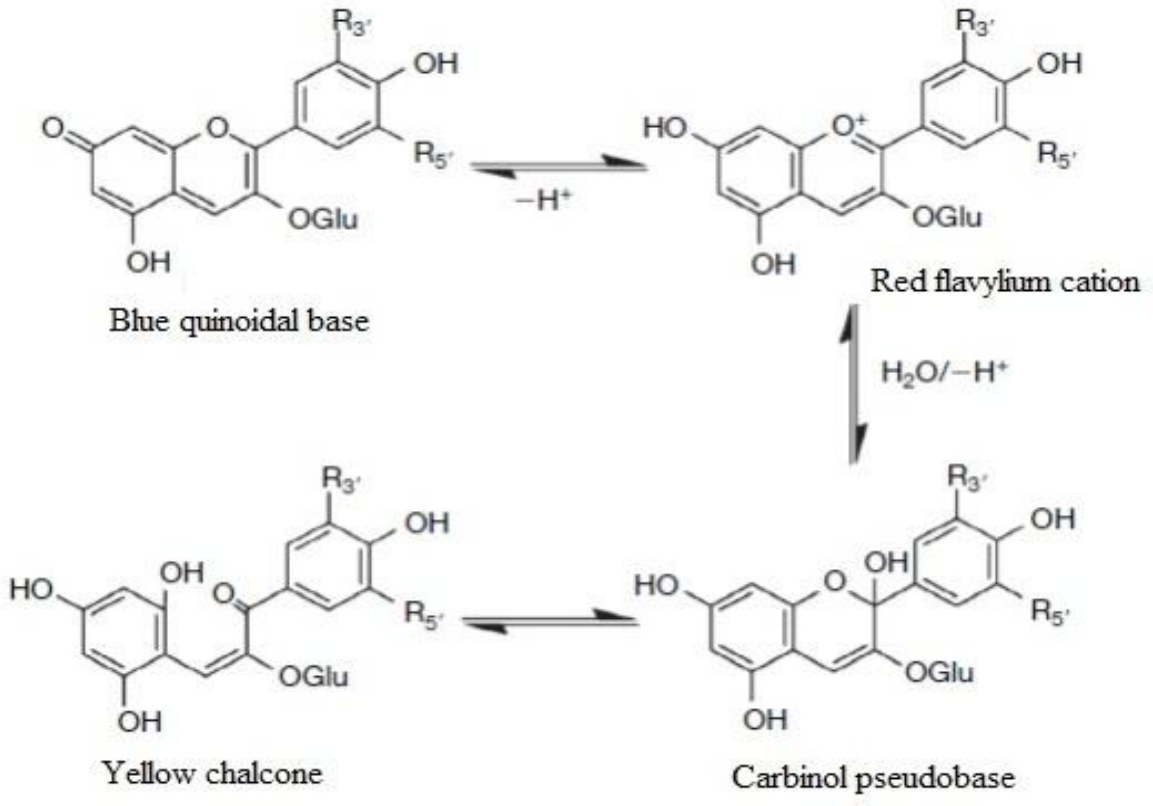

Fig. 2.2. Effect of pH on anthocyanin structure and resulting color changes (He and Giusti 2010).

When pH value increases beyond 7, degradation reactions take place which are dependent upon the substituent groups (Castañeda-Ovando et al. 2009). The phenomenon by which pigments and other colorless organic compounds and metallic ions form complex associations, resulting in change or increase in color intensities is known as copigmentation (Boulton 2001). Researchers have attributed the stability of pigments in plants to copigmentation reaction of anthocyanins with other compounds (Davies and Mazza 1993; Mazza and Brouillard 1990). Copigments are generally colorless but when they combine with anthocyanins, a

hyperchromic effect is produced which is accompanied with a bathochromic shift in the absorption spectra. Possible copigments can be other anthocyanins, metals, polysaccharides, amino acids or flavonoids. Metallic ion interaction with anthocyanins is another pathway for color stabilization in plant matter. Clifford (2000) attributed colors in flowers to the formation of chelates between metal and flavylium salts. Blue color in plant matter has been attributed to the formation of complexes between anthocyanins and metals such as Al, Fe, Cu and Sn (Starr and Francis 1973) or Mg or Mo (Hale et al. 2001). Pryoanthocyanins, a new class of pigments detected in red wine, result from reactions between anthocyanins and molecules with low molecular weights such as pyruvic acid and flavonols (Cameira-dos-Santos et al. 1996). Because of their superior stability at various pH values, they are also drawing considerable interest from researchers.

2.3.2. Stability of anthocyanins

Stability is an important consideration for a pigment when its application as a food colorant is discussed. Since all the anthocyanins contain a resonating flavylium nucleus, they are expected to be reactive. In general, 3-glucosides are highly stable as compared to their peonidin and malvinidin counterparts. Acylated anthocyanins show higher stability in neutral or weak acidic solutions (Brouillard 1982). The colors displayed by anthocyanins are highly dependent on the acidity of the solutions. At pH 4, the chalcone form is largely colorless; therefore, anthocyanins can only be used in solutions with pH values below 4. The effect of light on anthocyanins in general is deleterious. When exposed to light, acylated diglucosides in wine were more stable than monoglucosides while degradation was intermediate in nonacylated diglucosides (Van Buren et al. 1968). In another study, Palamidis and Markakis (1975) reported

that anthocyanins recovered from grape pomace and incorporated in a carbonated beverage displayed increased degradation due to light exposure.

Since heat treatment is an important part of food processing, heat effects on anthocyanins has been studied widely. As is the case with most of the natural pigments, there is a rapid decrease in amount of anthocyanin pigments at high temperatures. A logarithmic relationship between temperature and anthocyanin degradation was proposed and Markakis et al. (1957) recommended a short time/high temperature process for anthcyanin retention in processed foods. Interactions between ascorbic acid and anthocyanins are of particular interest because many fruit juices and beverages either contain vitamin C or are fortified with it. It has been observed that both react with each other; resulting in loss of appearance as well as nutritive value of the product (Markakis et al. 1957). Anthocyanins are highly reactive towards metal salts and form stable complexes. It has been reported that Cu, Fe and Al salts provided greater stability to anthocyanins in cranberry juice cocktail (Starr and Francis 1973). As a result, use of metal salts for anthocyanin stabilization has been proposed.

2.3.3. Health benefits associated with anthocyanins

Medicinal value of colored pigments for humans is a widely accepted doctrine in folk medicine. However, due to the recognition of potential health benefits of dietary polyphenols, interest in anthocyanins has increased greatly in recent times (Scalbert and Williamson 2000). Most widely suggested *modus operandi* of these pigments with respect to their intervention in human therapeutic targets is their free radical scavenging and antioxidant capabilities. The consumption of red wine containing anthocyanins is attributed to low incidence of heart disease in French populations, popularly called the French Paradox (Renaud and de Lorgeril 1992). Free radical scavenging activity and antioxidant properties of anthocyanins have been documented *in*

vitro and they have demonstrated even higher antioxidant activities than vitamin C and E (Bagchi et al. 1998; He and Giusti 2010). It has been reported that anthocyanins help in controlling cardiovascular disease (Andriambeloson et al. 1998; Folts 1998), demonstrate anti-carcinogenic and tumor inhibiting properties (Hou 2003; Meiers et al. 2001) and assist in fighting obesity and inflammation (Rossi et al. 2003; Tsuda et al. 2003).

However, since anthocyanins are just a part of wide range of other polyphenols present in fruits and vegetables, it is still an open question whether polyphenols work collaboratively or only anthocyanins are specifically responsible for health benefits (Seeram et al. 2004; Zhang et al. 2008). Several researchers have suggested that other mechanisms are equally likely to contribute (Tsuda et al. 1996; Tsuda et al. 2002; Wang and Jiao 2000).

2.3.4. Anthocyanin content in fruits, vegetables and cereals

Humans have been ingesting anthocyanins in fruits and vegetables since time immemorial. There are no reported studies on adverse impacts of oral consumption of anthocyanins in humans (Brouillard 1982). Colored fruits, vegetables and cereals such as berries, grapes, peaches, pomegranates, plums, red radishes, eggplants, purple sweet potatoes, purple corn and black rice are sources of anthocyanins in the human diet (Wu et al. 2006). There is a wide range of total anthocyanin distribution in fruits and vegetables with berries in general containing the maximum concentrations. Naczk and Shahidi (2004) attributed this variation to various environmental factors such as light, temperature and altitude. An earlier study estimated average per person daily intake of anthocyanins to be 180 to 215 mg (Kühnau 1976); however, in a recent study, Wu et al. (2006) estimated this amount to be only 12.5 mg in the US. Personal dietary habits play a big part in anthocyanin consumption; for example, one serving of blueberries can provide 500 mg anthocyanins while a bottle of red wine can contribute 200 mg

(Clifford 2000). Anthocyanin contents of various fruits, vegetables and cereals are summarized in Table 2.2.

TABLE 2.2. Anthocyanin contents of fruits, vegetables and cereals.

Source	Anthocyanin content (mg/kg)	Reference
Apple (peel)	100-2160 (fresh weight)	Eder (2000)
Bilberry	4600 (fresh weight)	Eder (2000)
Blackberry	830-3260 (fresh weight)	Mazza and Miniati (1993)
Black carrot	1820 (fresh weight)	Algarra et al. (2014)
Black currant	4760 (fresh weight)	Wu et al. (2006)
Black raspberry	6870 (fresh weight)	Wu et al. (2006)
Blueberry	250-4950 (fresh weight)	Mazza and Miniati (1993)
Blue corn	300 (dry basis)	Li et al. (2011)
Chokeberry	5060-10000 (fresh weight)	Clifford (2000)
Cranberry	250 (fresh weight)	Timberlake and Henry (1988)
Egg plant	7500 (fresh weight)	Clifford (2000)
Elderberry	13750 (fresh weight)	Wu et al. (2006)
Grape	60-6000 (fresh weight)	Mazza and Miniati (1993)
Plum	19-250 (fresh weight)	Timberlake and Henry (1988)
Purple corn	5990 (not mentioned)	Yang et al. (2009)
Purple sweet potato	840-1740 (fresh weight)	Bridgers et al. (2010)
Raspberry (black)	763-4277 (fresh weight)	Timberlake and Henry (1988)
Red cabbage	600-2000 (fresh weight)	Mazza and Miniati (1993)
Red radish (peel)	110-600 (fresh weight)	Giusti et al. (1998)
Strawberry	150-350 (fresh weight)	Timberlake and Henry (1988)
Onion	250 (fresh weight)	Timberlake and Henry (1988)

2.3.5. Current status of anthocyanin extraction for commercial use

As discussed above, synthetic dyes are potential health hazards and do not add any nutritive value to the foods, therefore, there is a need of natural alternates to replace them. For example, anthocyanins can be used as a replacement of FD&C Red 40 dye. Food supplement industry is another potential market for anthocyanins. However, economical anthocyanin recovery will be a determining factor if they are to supplant synthetic dyes from food industry. Currently, anthocyanins are extracted from purple colored fruits and vegetables using solvent extraction method. Acidified organic solvents are usually preferred for extraction due to the polar nature of anthocyanins (He and Giusti 2010). Industrial scale anthocyanin recovery from fruits and vegetables such as blueberries, blackberries and purple sweet potatoes require the entire material to be macerated followed by anthocyanin extraction using solvents. The main drawback of this process is that the remaining material, after anthocyanin extraction does not have much economic value. Another approach used for black carrots is to squeeze their juice and concentrate it using drying, which is energy intensive. In the case of grape pomace, one of the biggest sources of these pigments, anthocyanins are extracted using solvents and one kg dried pomace typically yields 1076 mg anthocyanins (Khanal et al. 2010). Therefore, not only greater inputs are required in terms of plant capacity, chemicals and material processing, at the same time processing residues have little economic value, rendering the process of anthocyanin recovery uneconomical. This has largely restricted wide scale adoption of anthocyanins as a replacement for FD&C Red 40 by the US food industry.

2.4. Colored corn for anthocyanin recovery

Keeping in mind the emerging natural color market and inherent drawbacks associated with the industrial anthocyanin recovery procedures in vogue, we intended to study the

suitability of colored corn cultivars as a potential source of anthocyanins for their economical recovery. Some colored corn cultivars such as purple and blue corn contain anthocyanin amounts comparable to many other well known sources of pigments (Table 2.2). In contrast to the others, corn can be abundantly grown, has greater shelf life and can be transported easily, giving it advantages over other anthocyanin sources. Our main research hypothesis was that the colored corn can be an economical source of anthocyanins and utilizing corn processing expertise available at University of Illinois at Urbana-Champaign, we can tailor the process to ensure that corn coproducts, after anthocyanin recovery, can be utilized further. This would yield significant economic advantages compared to other methods currently employed by the industry. Once an efficient process for economic anthocyanin recovery from corn is realized at lab scale, it can be implemented in the corn processing industry. Finally, the large scale of established corn wet milling, dry milling and dry grind industry in the US can be harnessed to economically recover anthocyanins and utilize the remaining corn fractions. The whole process will provide a very efficient and economical way of anthocyanin recovery and assist in replacement of FD&C Red 40 dye from many processed foods.

Chapter 3. Coproduct yields of purple, blue and yellow dent corn from various milling processes

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3.1. Introduction

In the year 2014, the US corn processing industry processed 47% of the 14.2 billion bushels of corn harvested. The dry grind industry alone utilized 5.4 billion bushels of corn producing 14.3 billion gallons of ethanol and 36 million metric tons of distillers dried grain with solubles (DDGS) used as animal food. The wet milling and dry milling industries together processed another 1.3 billion bushels (NCGA 2015; RFS 2015). Due to wide fluctuations in corn and coproduct prices, industry is looking for higher value coproducts (Rodriguez et al. 2010; Rosentrater 2007). Some value added coproducts that have been proposed are biodegradable plastics (Bothast and Schlicher 2005), zein (Dickey et al. 2001), corn fiber oil (Moreau et al. 1996), biodegradable cat litter (Vaughn et al. 2011) and furfural (Xiang and Runge 2014).

Some colored corn cultivars contain anthocyanins, which can be used as a natural alternative to chemical dyes in foods, cosmetics as well as dietary supplements. They belong to the class of flavonoids present in plant matter and are responsible for vivid colors expressed in flowers, vegetables, fruits and leaves. In addition to assisting plants in physiological functions, anthocyanins deter herbivores and parasites (Lev-Yadun and Gould 2008) and help in protecting plants from UV-radiation (Mazza and Miniati 1993). More than 635 anthocyanins, imparting blue, purple and red colors to plant matter, have been identified in nature (Anderson and Jordheim 2008).

High intake of anthocyanins can decrease occurrence of some chronic diseases. Red wine containing anthocyanins has been associated with low incidence of heart disease in French despite their high fat diet, popularly known as the French Paradox (Renaud and de Lorgeril 1992). Although a single class of compounds cannot account for all health benefits, anthocyanins in combination with other phytochemicals are known to be powerful antioxidants, help in preventing cardiovascular diseases, demonstrate anti-carcinogenic/anti-inflammatory activities and assist in controlling obesity and diabetes (He and Giusti 2010; Matsumoto et al. 2002; Zhang et al. 2008). Increasing health concern among customers and documented harmful behavioral and neurological effects of synthetic dyes on children (McCann et al. 2007) are forcing food industry personnel to look for natural food colorants to replace synthetic dyes. Wu et al. (2006) analyzed 24 different fruits, vegetables and nuts for anthocyanin contents and found that chokeberries, elderberries, black raspberries and blueberries contained 14.8, 13.7, 6.8 and 4.8 g/kg anthocyanins, respectively. Purple corn (*Zea mays L.)*, a colored cultivar of corn native to the Andes region of South America contains 6 g/kg anthocyanins (Yang et al. 2009). Blue corn (*Zea mays var. saccharata*) is reported to contain 0.3 g/kg anthocyanins (Li et al. 2011).

Currently anthocyanins are recovered mainly from purple colored fruits and vegetables such as blueberries, black carrots, sweet potatoes and dark colored grapes using solvents, therefore, the processing byproducts cannot be utilized further. Resulting high costs associated with anthocyanin recovery have limited their use in the food industry; there is an interest in exploring economical ways of recovery. In one study (Bridgers et al. 2010), using industrial purple fleshed sweet potatoes for anthocyanin extraction has been explored. Being relatively inexpensive, having longer storage life and with considerable anthocyanin content, colored corns can be used for their recovery and remaining fractions can be utilized. The large scale of the US

corn processing industry offers this unique opportunity. However, various milling properties of these anthocyanin containing corn varieties need to be ascertained and their suitability for conventional corn processes determined.

There is lack of research comparing various milling characteristics of purple and blue corn with respect to yellow dent corn. Using 1 kg scale procedures, wet milling, dry milling and dry grind properties of purple and blue corn were compared with yellow dent corn. Prime and coproduct yields from these corn types were compared.

3.2. Materials and Methods

3.2.1. Materials

Purple corn was procured from a specialty foods vendor (Lot No. L670106, Angelina's Gourmet, Swanson, CT), Jerry Peterson Blue Organic corn (Lot No. 41633) was purchased from Johnny's Selected Seeds (Fairfield, ME) while a high starch yielding cultivar of yellow dent corn (*Zea mays var. indentata*) was sourced from a major seed supplier. Moisture contents of purple, blue and yellow dent corn were 14.5, 16.2 and 12.5%, respectively. Corn was cleaned by using a 12/64" (4.8 mm) sieve for the removal of broken corn and foreign material (BCFM). For the wet milling process, corn moisture content was determined using 103°C air oven method (Approved Method 44-15A, AACC International 2010). Moisture of ground flour for dry grind process was determined with an air oven at 135°C for 2 hr (Approved Method 44-15A, AACC International 2010). Corn moisture content for the dry milling process was measured using an electronic moisture measurement device (GAC II, Dickey-John, Auburn, IL). Moisture contents of all process fractions were measured using a two stage oven method. Fractions were oven dried overnight at 49°C and moisture content of dried samples were ascertained using an air oven at 135°C for 2 hr (Approved Method 44-19, AACC International 2010).

Corn kernel physical properties were determined using standard procedures. Test weight (TW) used specified apparatus according to standard method (Approved Method 55-10, AACC International 2010) and was expressed in kilograms per hectoliter (kg/hL). Absolute density of corn was measured using the ethanol column test as described in Hill et al. (1990). Thousand-kernel weight (TKW) was measured using the procedure outlined by Groos et al. (2003). All physical properties were measured in triplicates.

Compositional analyses of the corn types included crude protein content (Method 990.03, AOAC 2003), crude fat content (Method 920.39, AOAC 2003) and neutral detergent fiber content (Van Soest et al. 1991) at a commercial analytical laboratory (Illinois Crop Improvement Association, Champaign, IL). Starch content was measured using acid hydrolysis method described by Vidal et al. (2009). All analyses were done in duplicate.

3.2.2. Milling processes

3.2.2.1. Wet milling process

For wet milling of corn, 1 kg scale wet milling procedure described by Eckhoff et al. (1993) was modified. Corn (1 kg samples) was steeped in 2 L water, 0.5% lactic acid and 0.2% sulfur dioxide for 24 hr at 52°C using batch steeping conditions. Post steeping, steepwater was measured by using 2000 mL graduated cylinder. Steeped corn with 2 L water was ground using a blunted blade Waring commercial grade blender (Dynamic Corp. of America, New Hartford City, CT) for 5 min at 4500 RPM and 500 mL water was used to clean the blender jar. Slurry and germ skimming was done in a 10 L basket using 14 and 18 mesh stainless steel screens. Germ was rinsed with 1 L water over 1 mm round hole screen for the removal of starch and fiber from germ. Degerminated slurry was ground fine using a Quaker City plate mill (Model 4-E, The Straub Co., Hartboro, PA); 1 L water was used to wash the mill and bucket. Finely ground slurry

was allowed to settle for 30 min and 2 L water was decanted. Slurry was poured on a vibrating screen of 270 mesh for separating fiber. Fresh water (4 L) and 2 L decanted water was used to wash the fiber of starch and gluten. The starch-gluten slurry was allowed to settle for 30 min and 4 L water was decanted before separating starch and gluten according to the tabling procedure described in Eckhoff et al. (1993). Fresh water used for starch tabling was 2 L for purple corn compared to 1 L for blue and yellow dent corn. Starch was allowed to dry on the table overnight and scraped the next day. After drying over night in 49°C oven, percentages of gluten dry matter and steep solution were determined using an air oven at 135°C for 2 hr (Approved Method 44-19, AACC International 2010). Fiber, germ and starch fractions were also dried over night in 49°C oven and moisture contents were determined using air oven at 135°C for 2 hr (Approved Method 44-19, AACC International 2010).

3.2.2.2. Dry milling process

One kg corn samples were dry milled employing a modified lab scale dry milling procedure with single stage tempering reported by Rausch et al. (2009). Sieved fractions (+ and -5) were passed through roller mill twice rather than four times. Corn kernel moisture was tested using an electronic moisture tester and requisite amount of water was calculated to increase moisture content to 23.5% (wet basis). Corn kernels and water were tempered in 4 L plastic containers which rotated continuously for 20 min on horizontal axis at 0.5 rpm. Increased moisture corn was passed through a lab scale horizontal drum degerminator; resulting fractions were placed in a convection oven at 49°C for 2 hr. Conditioned fractions were sifted for 3 min over 5 mesh screen in a box sifter (Model 130-11, Great Western, Leavenworth, KS). The +5 and -5 fractions were roller milled in a lab scale roller mill (Allis Chalmers, Appleton, WI) twice. The +5 fractions were sieved on a 10 mesh screen and +10 fractions were collected as

germ and pericarp while -10 fractions as large grits. The -5 fractions were also sieved on a 10 mesh screen. The +10 fractions were collected and added to germ and pericarp while -10 fractions constituted small grits and fines. The -10 fractions were sieved further on 24 mesh screen, +24 fractions constituted small grits while -24 fractions were collected as fines. Germ and pericarp were separated by using a lab scale aspirator (Model 6DT4, Kice Metal Products, Wichita, KS). Fraction moisture contents were determined with an air oven at 135°C for 2 hr (Approved Method 44-15A, AACC International 2010).

3.2.2.3 Dry Grind process

One kg cleaned corn was ground with a laboratory hammer mill at 500 rpm using a 0.5 mm sieve (1100 W, model MHM4, Glen Mills, Clifton, NJ). Finely ground corn was mixed with DI water to obtain corn slurry having 29% solids on dry basis (db). To adjust slurry pH to 5.8 for liquefaction, sulfuric acid (10*N*) was used. For hydrolyzing starch, 250 µL of α-amylase enzyme (SPEZYME® CL) sourced from DuPont Industrial Biosciences (Palo Alto, CA) with a declared activity of 15,225 alpha amylase units (AAU)/g was added to the slurry. Contents were poured in 3 L flasks (model DHOD-182, Bellco Glass, Vineland, NJ) which were put in a water bath for 90 min at 84°C with an agitation of 100 rpm using an overhead drive. Post liquefaction, slurry pH was adjusted to 4.2 using 10*N* sulfuric acid. For simultaneous saccharification and fermentation, 850 µL of glucoamylase enzyme (DISTILLASE® SSF) obtained from DuPont Industrial Biosciences (Palo Alto, CA) with a minimum declared activity of 380 glucoamylase units (GAU)/g was added. Urea solution (4 mL, 50% w/v) prepared with urea (99.6% ACS grade, Fisher Scientific, Fair Lawn, NJ) was added as a nitrogen source in the slurry. Yeast used in fermentation was active dry yeast (Ethanol Red, Fermentis, Lesaffre Yeast, Milwaukee, WI). Yeast culture was prepared by mixing 5 g yeast in 25 mL distilled water and kept in an incubator

at 32°C for 20 min with agitation. Yeast culture (20 mL) was added to the 3 L vessels and fermentation was done for 72 hr at 32°C.

Fermentation samples (5 mL) were collected at 0, 2, 4, 18, 24, 48 and 72 hr and centrifuged at 4,000 rpm (IEC CL 30, Thermo Electric Corporation, West Chester, PA). Liquid was passed through a 0.2 μm syringe filter to 1 mL vials, the resultant liquid was passed through an ion-exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) and separated sugars, organic acids and alcohols were quantified using a refractive index detector (model 2414, Waters Corporation, Milford, MA). A refractive index detector was employed to determine ethanol concentrations and data ware analyzed using HPLC software (Model 2414, Waters Corporation). After 72 hr fermentation, remaining mash was kept in 90°C water bath for 2 hr to evaporate ethanol. Remaining whole stillage was poured in an aluminum pan and kept in 49°C oven for 3 days. Moisture content of DDGS was determined using an air oven at 135°C for 2 hr (Approved Method 44-15A, AACC International 2010). Theoretical ethanol yields (L/kg) were calculated based upon corn standard bushel weight of 56 lb/bu while total starch content of each corn type was determined using acid hydrolysis method (Vidal et al. 2009). Actual ethanol yields (L/kg corn db) were determined by final ethanol concentrations. Conversion efficiencies were calculated from the ratios of actual and theoretical ethanol yields.

3.2.3. Hunter color parameters

Starch samples from wet milling were analyzed for their color using Hunter Lab Colorimeter (model Chroma Meter CR-300, Konika Minolta Inc., Japan). Parameters ascertained were *L* value (0 for black and 100 for white), *a* value (+ for red and – for green) and *b* value (+ for yellow and – for blue). A standard white tile provided with the device was used for reference. Whiteness value was calculated using the formula:

$$W = 100 - [(100 - L)^2 + (a)^2 + (b)^2]^{1/2}$$

3.2.4. Statistical analysis

Each process was replicated three times. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests were conducted using SAS Studio (SAS Institute, Cary, NY). The level selected to detect difference among means was 5% ($p < 0.05$).

3.3. Results and Discussion

3.3.1. Physical, compositional and Hunter color characteristics

Physical and compositional characteristics of three corn types are summarized in Table 3.1. TKW for purple and yellow dent corn was similar while lower for blue corn. For purple corn, TKW varied between 354.8 to 361.7 g with a mean value of 359.2 g. Blue corn TKW varied between 297.2 to 328.3 g with a mean value of 313.5 g. TKW for yellow dent corn varied between 346.2 to 364.2 g with a mean value of 355.1 g. TKW values for all three corn types were greater than mean TKW of 308.5 g for Germplasm Enhancement of Maize (GEM) varieties as reported by Singh et al. (2001) and 297.6 g as reported by Taboada-Gaytan et al. (2009) and corresponded well with the mean TKW for commercial hybrids as reported by Singh et al. (2001). Higher TKW is a preferred wet milling characteristics, it is associated with greater starch and protein yield and lesser yields of fiber (Serna-Salvidar 2012).

TW of purple corn varied from 72.3 to 73.0 kg/hL with a mean value of 72.6 kg/hL, for blue corn TW varied from 87.2 to 87.6 kg/hL with a mean value of 87.5 kg/hL and TW for yellow dent corn varied from 88.9 to 89.3 kg/hL with a mean value of 89.1 kg/hL. TW for purple corn was on the lower side of GEM lines (mean TW of 79.3 kg/hL) as reported by Singh et al. (2001) and range of 73.5 to 80.4 kg/hL as reported in Taboada-Gaytan et al. (2009). Mean TW

TABLE 3.1. Physical and compositional corn characteristics.

	Physical characteristics			Compositional characteristics (% db)			
	Thousand-kernel weight (g)	**Test weight (kg/hL)**	**Absolute density (g/mL)**	**Starch**	**Crude protein content**	**Crude oil content**	**Neutral detergent fiber**
Purple corn	359.2 ± 0.31a	72.55 ± 0.30c	1.06 ± 0.02c	63.41 ± 0.17c	10.31 ± 0.20b	4.74 ± 0.03b	9.44 ± 0.18a
Blue corn	313.5 ± 12.75b	87.46 ± 0.17b	1.19 ± 0.01b	66.54 ± 0.12b	11.16 ± 0.08a	5.69 ± 0.04a	9.70 ± 0.04a
Yellow dent	355.1 ± 0.73a	89.08 ± 0.18a	1.23 ± 0.01a	75.21 ± 0.21a	8.44 ± 0.04c	3.28 ± 0.01c	8.03 ± 0.17b

Physical characteristics: Mean ± SD from three replicates; Compositional characteristics: Mean ± SD from two replicates; Means followed by the same letter in a column are not different ($p < 0.05$).

for yellow dent corn and blue corn were 89.1 and 87.5 kg/hL, respectively, and higher than those reported for earlier studies of GEM lines (Singh et al. 2001; Taboada-Gaytan et al. 2009). TW is used in determining corn grade and influences corn selling price in the market (Paulsen et al. 2003). All three corn types used in this study had test weights greater than 69.5 kg/hL required for US Grade No. 2 corn.

Absolute density is used to determine kernel hardness which is relative proportion of vitreous to floury endosperm in the corn kernel (Correa et al. 2002). Absolute density is important in determining the suitability of a corn type either for wet milling or dry milling. A corn cultivar containing softer endosperm facilitates easier extraction of starch due to weaker protein matrix around starch granules and therefore is suited better for wet milling (Watson 1987); whereas, a corn type with harder endosperm is more suitable for dry milling as it tends to yield grits with larger sizes. In this study, purple corn absolute density varied from 1.037 to 1.076 with mean value of 1.06 g/mL, blue corn absolute density varied from 1.172 to 1.204 with a mean value of 1.18 g/mL. Absolute density of yellow dent corn varied from 1.229 to 1.235 with a mean value of 1.23 g/mL. Absolute densities for three corn types were different from each other and lesser than reported mean values of 1.301 and 1.272 g/mL for exotic and adapted lines (Taboada-Gaytan et al. 2009) and a mean of 1.320 g/mL for adapted inbred lines and exotic accessions as reported by Singh et al. (2001).

Starch content was largest in yellow dent corn at 75.2% followed by blue and purple corn at 66.5 and 63.4% (db), respectively. Blue corn had the largest crude protein content 11.2% while respective values for purple and yellow dent corn were 10.3 and 8.4% (db), respectively. Crude oil contents for blue, purple and yellow dent corn were 5.7, 4.7 and 3.3% (db), respectively. Colored corns had greater neutral detergent fiber contents with blue and purple corn

having 9.7 and 9.4%, respectively, while yellow dent corn had 8.0% (db). Starch is the main product of interest in wet milling and its whiteness is a desired quality characteristic (Wang et al. 2000). For purple corn starch, mean L value, mean a value (an indicator of redness or greenness) and mean b value (an indicator of blueness and yellowness) were 82.03, 7.37 and -0.26, respectively (Table 3.2). Mean L, a and b values for blue corn starch were 91.45, 4.71 and -1.49 while corresponding values for yellow dent corn starch were 97.70, -2.89 and 9.23, respectively.

TABLE 3.2. Hunter color characteristics of starches.

	Purple corn	**Blue corn**	**Yellow dent corn**
L value	82.03 ± 2.04c	91.45 ± 0.16b	97.70 ± 0.28a
a value	7.37 ± 0.73a	4.71 ± 0.18b	-2.89 ± 0.07c
b value	-0.26 ± 0.19b	-1.48 ± 0.15c	9.23 ± 0.22a
Whiteness	80.58 ± 2.17b	90.11 ± 0.04a	90.05 ± 0.26a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$).

Purple corn starch displayed redness due to positive a values which indicated that starch contained some anthocyanins. Mean whiteness value for purple corn was 80.58. Corresponding mean whiteness value for blue corn and yellow dent corn were 90.11 and 90.05, respectively. Whiteness values for blue and yellow dent corn were similar while different from whiteness value of purple corn. Wang et al. (2000) suggested that Hunter L values of greater than 90 are acceptable values for starches; in this study purple corn starch had a mean L value of 82.03 which means further refining will be required to improve purple corn starch whiteness.

3.3.2. Wet milling characteristics

Starch is the most important product of the wet milling process and high starch yield in general determines the millability of a particular type of corn. The ease with which starch can be extracted depends upon composition of endosperm and relative strength of protein matrix binding starch granules (Eckhoff et al. 1996; Eckhoff et al. 1993; Watson 1987). Wet milling fraction yields of purple, blue and yellow dent corn on dry basis are summarized in Table 3.3. Mean starch yield for purple corn and blue corn were was 63.4 and 61.5% (db), respectively and were different as compared to yellow dent corn with a mean starch yield of 70.13% (db). Starch yield for purple corn was similar to 64.8% reported by Eckhoff et al. (1993) but less than mean industrial yields of 67.5% reported by Johnson and May (2003). Blue corn starch yield was less than those reported in both studies. Yellow dent corn starch yield had a mean value of 70.1% and was greater than those reported by other lab-scale and industrial processing studies (Eckhoff et al. 1993; Johnson and May 2003; Steinke and Johnson 1991).

Purple corn and blue corn starch yields corresponded well with the results of 100 g lab-scale mean starch yields of 62.4% for hybrid grain of exotic introgressed lines and commercial adapter testers (Taboada-Gaytan et al. 2010) while yellow dent corn starch yields were higher. Yellow dent corn yielded 6.7% more starch than purple corn while blue corn yielded 1.9% less starch compared to purple corn. Mean germ yields for purple, blue and yellow dent corn were 6.2, 8.0 and 6.1%, respectively and blue corn germ yield was different from other two. Germ yields for purple and yellow dent corn were less than those reported for other studies and industry while blue corn germ yields were greater (Table 3.3). Purple corn and blue corn fiber yields were similar at 10.9 and 10.6% (db), respectively, and were different as compared to yellow dent corn at 8.1% (db).

Purple and blue corn contained 2.8 and 2.5% more fiber (db), respectively, as compared

to yellow dent corn (Table 3.4). Mean gluten recoveries for purple and blue corn were 13.7 and 14.2% (db), respectively, and higher than for yellow dent corn at 10.5% (db). Higher gluten

TABLE 3.3. Wet milling fraction yields of different corn (% db).

	Purple corn	**Blue corn**	**Yellow dent corn**
Starch	63.36 ± 0.17b	61.46 ± 0.43c	70.13 ± 0.01a
Germ	6.19 ± 0.26b	8.00 ± 0.06a	6.13 ± 0.03b
Fiber	10.88 ± 0.24a	10.58 ± 0.14a	8.15 ± 0.06b
Gluten	13.77 ± 0.47a	14.23 ± 0.01a	10.54 ± 0.01b
Steepwater solids	5.34 ± 0.02a	5.18 ± 0.07b	4.15 ± 0.03c
Total solids	99.54 ± 0.41a	99.45 ± 0.25a	99.09 ± 0.06a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$).

values for purple and blue corn (3.2 and 3.7% db, respectively) compared to yellow dent corn could be attributed to poor starch-gluten separation due to stronger binding of starch granules by protein matrix (Watson 1987). Steepwater solids yields for purple and blue corn were (5.3 and 5.1% db, respectively) and different from yellow dent corn (4.1% db). However these yields were less than mean steep water solids yield of 7.3% (db) as reported in previous 1 kg scale wet milling studies (Eckhoff et al. 1993; Steinke and Johnson 1991) and 7.6% (db) for industry as reported by Johnson and May (2003).

This can be attributed to longer steep times ranging from 30 to 48 hr in above studies; whereas, in this study steeping duration was 24 hr. However, steep water solids yield for yellow

TABLE 3.4. Comparison with other wet milling studies (% db).

	Steinke and Johnson (1991)	Eckhoff et al. (1993)	Johnson and May (2003)	Current study		
				Purple corn	Blue corn	Yellow dent
Starch	58.4	64.8	67.5	63.4	61.5	70.1
Germ	6.6	7.0	7.5	6.2	8.0	6.1
Fiber	19.2	9.9	11.5	10.9	10.6	8.1
Gluten	8.9	9.9	5.7	13.8	14.2	10.5
Steepwater solids	7.6	7.0	7.6	5.3	5.1	4.2
Total solids	100.3	98.6	99.1	99.5	99.4	99.1
Steep time, hr	48	36	30-36	24	24	24

dent corn corresponded well with mean steep water solid yields of 4.2% (db) reported for 100 g wet milling studies by Dowd (2003). Total solids recovery for purple and blue corn was not different from total solids recovery for yellow dent corn at 99.1% (db). Results for all corn types were similar to those reported in earlier studies (Table 3.4). Purple and blue corn can be used for starch recovery using wet milling procedure; however, starch washing processes for purple corn need to be optimized to clear final starch of reddish hue due to presence of anthocyanins.

3.3.3. Dry milling characteristics

The main purpose of dry milling of corn is to break corn kernel into its three main compositional parts, namely endosperm, germ and pericarp. The main coproduct in dry milling is large sized flaking grits which are used in making breakfast cereals. The larger the grit size, greater is the market value, since grit size can be reduced but not vice-versa (Rausch et al. 2009; Yuan and Flores 1996). Hardness of corn kernel partially determines flaking grits yields as harder endosperm generally results in larger yield of flaking grits (Paulsen and Hill 1985). In this study, purple and blue corn yielded 21.8 and 24.4% (db), respectively large grits while large grits yield for yellow dent corn was 24.4% (db) (Table 3.5). Large grit yield of yellow dent corn and blue corn were similar while lesser for purple corn. Small grit yields for purple, blue and yellow dent corn were 22.2, 24.6 and 19.4% (db), respectively. Fines constituted largest proportion of coproducts for the three corn types at 36.1, 28.1 and 40.9% (db) for purple blue corn and yellow dent corn, respectively.

Fines yield was maximum for yellow dent corn and minimum for blue corn. Total endosperm fraction was 80.1% (db) for purple corn, 77.0% (db) for blue corn and 84.4% (db) for yellow dent corn. For purple and yellow dent corn, yield of small grits and fines was 60.0% (db), for blue corn yield was 53.0% (db). Purple, blue and yellow dent corn constituted of higher

amount of soft endosperm compared to harder endosperm (Table 3.5). Due to higher percentage of soft endosperm, which resulted in greater yields of small grits and fines during dry milling, both purple and blue corn were found to be better suited for wet milling or dry grind processes compared to dry milling. Purple corn had a pericarp yield of 9.9% (db), blue corn pericarp yield was highest at 12.5% (db) while yellow dent corn pericarp yield was least at 6.8% (db). Germ yields for purple and blue corn were similar at 9.4 and 9.5% (db), respectively, while it was least for yellow dent corn at 7.6% (db). Overall solids recovery was 99.5, 99.1 and 99.3% (db) for purple, blue and yellow dent corn, respectively.

Our results were different from those reported by Rausch et al. (2009), in which 11 hybrids of corn were dry milled. Mean yields reported for large grits, small grits, fines, total solids, germ and pericarp were 39.2, 25.2, 13.8, 78.2, 14.3 and 6.8% (db), respectively. Rausch et

TABLE 3.5. Dry milling fraction yields of different corn (% db).

	Purple corn	**Blue corn**	**Yellow dent corn**
Large grits	21.81 ± 0.06b	24.40 ± 0.45a	24.44 ± 0.35a
Small grits	22.22 ± 0.24b	24.57 ± 0.20a	19.42 ± 0.25c
Fines	36.13 ± 0.21b	28.08 ± 0.49c	40.93 ± 0.83a
Total endosperm	80.16 ± 0.10b	77.06 ± 1.03c	84.87 ± 0.38a
Pericarp	9.94 ± 0.05b	12.50 ± 0.33a	6.87 ± 0.21c
Germ	9.38 ± 0.27a	9.54 ± 0.31a	7.64 ± 0.41b
Total solids	99.47 ± 0.40a	99.09 ± 0.54a	99.29 ± 0.17a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$).

al. (2009) estimated starch content of the yellow dent corn to be 82 to 87 g of starch/100 g corn kernel; results for yellow dent corn with a mean endosperm yield of 84.9 % (db) are closer to that number. However, yield differences in large/small grits and fines can be attributed to relative differences of structure and composition of corns used in both studies.

3.3.4. Dry grind characteristics

With 200 operational plants in US, dry grind industry is a major processor of corn producing ethanol and DDGS. After 72 hr of fermentation, final ethanol concentrations for purple, blue and yellow dent corn were 14.5, 14.4 and 17.2% (v/v), respectively, as shown in Table 3.6. Wang et al. (2005) starting with 24.5% dry solids content reported ethanol concentration for conventional dry grind process at 14.2% (v/v) while Khullar et al. (2009) with 25% initial solids reported this value to be 14.1% (v/v), respectively. In this study, final ethanol concentration of purple and blue corn at 14.5 and 14.4 % (v/v), respectively, was comparable to the abovementioned works while ethanol concentration for yellow dent corn was higher due to higher solid loading at 17.2% (v/v). Typically in dry grind process, one third of corn starting material is converted to DDGS after fermentation. Wang et al. (2005) reported DDGS yield for conventional dry grind process to be 28.3% (db). In this study, mean DDGS yields for purple, blue and yellow dent corn were 41.6, 38.0 and 33.0% (db), respectively.

Highest DDGS yield was for purple corn while yellow dent corn yielded least DDGS (Table 3.6). Final ethanol concentration for yellow dent corn was 2.7% (v/v) greater than final ethanol concentrations for purple and blue corn implying higher starch concentration in former. Ethanol yields for yellow dent, purple and blue corn were 0.45, 0.35 and 0.33 L/kg,

TABLE 3.6. Dry grind characteristics of different corn.

	Purple corn	Blue corn	Yellow dent corn
Ethanol concentration (% v/v)	14.51 ± 0.28b	14.33 ± 0.06b	17.16 ± 0.11a
DDGS (% db)	41.59 ± 0.62a	37.98 ± 1.28b	32.94 ± 0.15c
Ethanol yield (L/kg)	0.35 ± 0.01b	0.33 ± 0.01c	0.45 ± 0.01a
Conversion efficiency (%)	76.54 ± 1.71b	69.68 ± 0.32c	84.36 ± 0.67a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$).

respectively. Ethanol conversion efficiency was highest for yellow dent corn at 84.4% while corresponding values for purple and blue corn were 76.5 and 69.7%, respectively.

Ethanol concentrations after 18 hr of fermentation were higher for purple and blue corn at 9.6 and 9.3% (v/v) in comparison to yellow dent corn 8.7% (v/v) (Fig. 3.1). However, after 24 hr, ethanol concentration of yellow dent corn became higher than the ethanol concentrations of purple and blue corn due to greater starch concentration in the former. Initial glucose levels for purple, blue and yellow dent corn were 1.1, 1.9 and 1.0% (w/v), respectively.

After 2 hr, glucose levels were 13.3 and 12.3% (w/v), respectively, for purple and blue corn while this was much higher at 20.0% (w/v) for yellow dent corn. After 18 hr of fermentation these levels were 3.6, 4.0 and 7.4% (w/v), respectively. After 48 hr, glucose concentrations for all corn types became negligible (Fig. 3.2). With final ethanol concentrations reaching 14.5 and 14.4% (v/v), respectively, both purple and blue corn can be used as an alternate feedstock for corn dry grind industry.

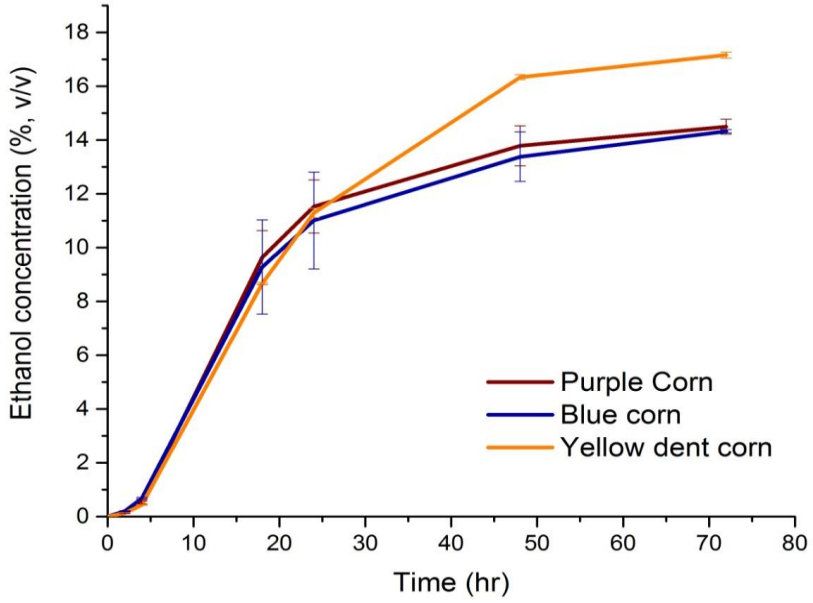

Fig. 3.1. Respective ethanol concentrations during fermentation. Error bars are ± 1 standard deviation.

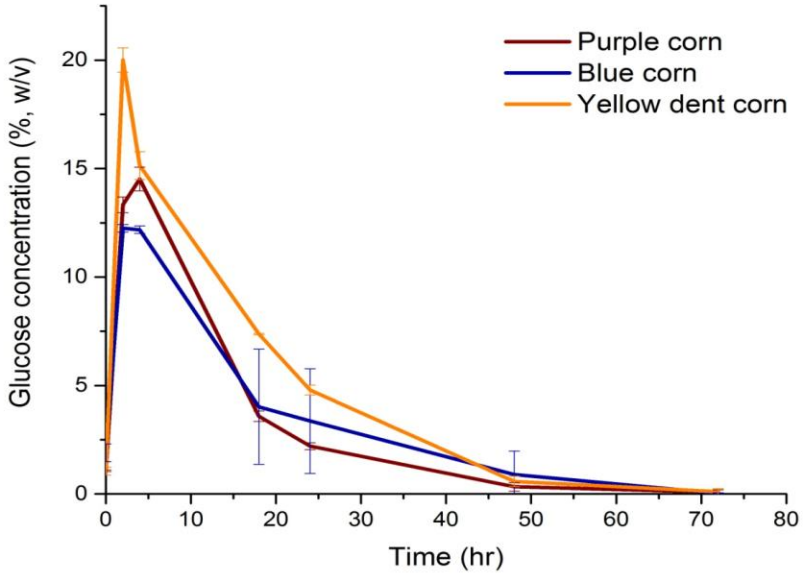

Fig. 3.2. Respective glucose concentrations during fermentation. Error bars are ± 1 standard deviation.

3.4. Conclusions

Wet milling, dry milling and dry grind characteristics of purple and blue corn were compared with yellow dent corn using 1 kg scale procedures. In wet milling, starch yields for purple, blue and yellow dent corn were 63.4, 61.5 and 70.1% (db), respectively. Purple corn had higher yields of fiber and steepwater solids while germ and gluten yields were greatest in blue corn. Purple and blue corn can be used as feedstock for wet milling industry. Whiteness value for blue corn starch was comparable with yellow dent corn starch but purple corn starch displayed red coloration and will require further refining. In dry milling process, large grits yield for purple, blue and yellow dent corn were 21.8, 24.4 and 24.4% (db), respectively, implying all corn types had dominant softer endosperm composition. Yellow dent corn contained largest total endosperm fraction at 84.5% as compared to 80.1% for purple corn and 77.0% for blue corn on dry basis. Blue corn had greater pericarp and germ yields as compared to the other two corn types. In dry grind process, purple corn yielded largest amount of DDGS (41.6%) while yellow dent corn yielded minimum at 32.9% (db). Final ethanol concentrations after 72 hr for purple, blue and yellow dent corn were 14.5, 14.4 and 17.2% (v/v), respectively. Overall ethanol conversion efficiency was highest for yellow dent corn at 84.4% while it was 76.5 and 69.7% for purple and blue corn, respectively. Final ethanol concentrations for both purple and blue corn were within industrially acceptable levels. Purple and blue corn can be used for anthocyanin recovery and utilized in all three processes.

Chapter 4. Distribution and yield of purple and blue corn anthocyanins in coproducts of wet milling, dry milling and dry grind processes

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4.1. Introduction

Anthocyanins, sourced from fruits, vegetables and cereals, can be used as a replacement of FD&C Red 40 dye from the food and cosmetic industry. However, higher costs associated with anthocyanin extraction have prevented large scale adoption by the food industry in the US. This is despite use of coal tar based FD&C Red 40 dye in foods is eliciting increased criticism from customers due to its potential link to attention-deficit/hyperactivity disorder (ADHD) in children. Therefore, there is an interest from food industry personnel in exploring cost effective ways to source natural red color.

Corn is the most important food grain in the US, accounting for more than 95 percent of total food grain production and use (USDA 2016). Out of 13.6 billion bushels of corn produced in the US in 2015, 1.7 billion bushels were exported and 44.8% of the remaining was utilized by the corn processing industry (NCGA 2015). The dry grind industry was the largest processor of corn (34.6%), producing fuel ethanol as the primary product and distillers dried grains with solubles (DDGS) as a coproduct. DDGS is used mainly as food for ruminants. The wet milling industry is another major processor (8.5%), producing starch as the primary product and corn gluten meal, corn gluten feed, corn germ and fiber as coproducts (NCGA 2015). Corn starch is processed further to produce high fructose corn syrup, modified starches and sweeteners. The wet milling industry produces diverse products, ranging from antibiotics to adhesives and amino

acids to edible gums (CRA 2016). The dry milling industry is the smallest amongst the three, processing only 1.7% of total US corn. The primary product from corn dry milling is large grits. Coproducts include small grits, fines, germ and pericarp. Large grits are used for making corn flakes and cereal bars while coproducts are utilized in brewing and in making corn flour and hominy feed (CRA 2016).

Colored corn types are rich sources of anthocyanins. These contain a wide range of total anthocyanins ranging from 51 mg cyanidin-3-glucoside equivalent (C3G)/kg in red corn (Abdel-Aal et al. 2006) to 5990 mg C3G/kg in purple corn (Yang et al. 2009). Other well known sources of anthocyanins include raspberry with 500 mg C3G/kg, blackberry containing 1500 mg C3G/kg (Pantelidis et al. 2007) and black rice containing 3300 mg C3G/kg (Abdel-Aal et al. 2006). Blue corn contains 300 mg C3G anthocyanins per kg corn (Li et al. 2011). Abdel-Aal et al. (2006) studied the anthocyanin distribution in different fractions of cereal grains such as black rice, red rice and blue wheat. However, the anthocyanin concentrations of different corn milling fractions were not reported. Large scale cultivation of corn, its long shelf-life and extent of corn processing industry in the US makes colored corn an attractive candidate for economical anthocyanin extraction.

Our objective was to ascertain the distribution and yield of purple and blue corn anthocyanins in various coproduct streams of wet milling, dry milling and dry grind processes to identify coproducts containing maximum concentrations of anthocyanins. Corn coproducts from these three processes were analyzed for monomeric anthocyanin concentration, calculated as mg of C3G equivalent and anthocyanin profile.

4.2. Materials and Methods

4.2.1. Materials

Purple corn (*Zea Mays L.)* was procured from specialty foods vendor (Lot No. L670106, Angelina's Gourmet Swanson, CT). Jerry Peterson Blue organic corn (*Zea mays var. saccharata)* was purchased from an online vendor (Lot No. 41633, Johnny's Selected Seeds, Fairfield, ME). Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (St. Louis, MO).

4.2.2. Milling processes

One kg scale milling processes were employed in triplicates for corn milling. Coproduct moisture contents were determined with an air oven at 135°C for 2 hr (Approved Method 44-15A, AACC International 2010). For wet milling, an adapted 1 kg scale procedure developed by Eckhoff et al. (1993) was used. One kg scale dry milling procedure developed by Rausch et al. (2009) was used for dry milling. For dry grind processing of corns, a 1 kg scale procedure used by Khullar et al. (2009) was employed.

4.2.3. Extraction of anthocyanins from corn coproducts

For the dry milling process, the coproducts analyzed were pericarp, large grits, small grits, fines and germ. For wet milling, steepwater, gluten slurry, gluten, starch and germ were analyzed. The only coproduct of dry grind process, DDGS, was analyzed. Solid coproducts were ground using a Kitchen-Aid coffee grinder for 25 sec. Ground material was passed through a 35 mesh sieve and material that did not pass through the sieve was ground for another 25 sec and passed through the same sieve. Material passing through the sieve were combined and used for extraction. Ground material (0.5 g) was suspended in 20 mL (40:1 liquid-to-solid ratio) 2% aqueous formic acid and stirred for 2 hr at room temperature. The suspension was filtered with Grade 1 Whatman® filter paper and the resulting filtrate was used to determine monomeric anthocyanins, polyphenols, tannins, color and for conducting HPLC/MS-MS analysis. After first

extraction, coproducts were added into 20 mL 2% formic acid and stirred at room temperature for 2 hr. The suspension from the second extraction was filtered and the filtrate was collected for further measurements. Third sequential extraction was conducted by mixing each one of the coproducts with 20 mL 2% formic acid and 25% ethanol to extract the anthocyanins. The mixture was stirred at room temperature for 2 hr and the filtrate was collected.

4.2.4. Measurement of monomeric anthocyanin concentration

Analysis for monomeric anthocyanin concentration (MA) was performed by the pH differential method using a microplate reader method in three independent replicates (Lee et al. 2005). Extracts obtained previously or aqueous coproducts from the wet milling process such as steepwater and gluten slurry were diluted using two buffers (pH 1.0, 0.25 M KCl and pH 4.5, 0.40 M sodium acetate). Two hundred µL diluted solutions at each pH were transferred to a 96 well plate and absorbance was read at 520 and 700 nm using a multi-well plate reader (Synergy 2, BioTek, Winooski, VT). Monomeric anthocyanin concentration was calculated as mg of cyanidin-3-glucoside (C3G) equivalents per L as described below:

$$MA\ (mg/L) = (A * MW * D * 1000)/(\varepsilon * PL * 0.45)$$

Where: A = (A520 – A700) at pH 1.0 – (A520 – A700) at pH 4.5; MW = 449.2 g/mol for C3G; D = dilution factor; PL = constant path length 1 cm; ε = 26900 L/mol·cm which is the molar extinction coefficient for C3G, 1000 was conversion factor from grams to milligrams and 0.45 was conversion factor from the established method to the plate reader method. Final results were expressed as mg C3G equivalents per g coproduct. Gluten slurry, a wet milling coproduct, was filtered and the filtrate was used for anthocyanin concentration determination directly.

4.2.5. Measurement of polyphenol concentration

Polyphenol concentrations were measured using the Folin-Ciocalteu method adapted to a microassay (Bower et al. 2014). Filtrates from the first formic acid extractions were diluted with deionized water; 15 µL of the diluted samples, standard or blank (deionized water) were added to a 96-well plate with 50 µL of 1N Folin-Ciocalteu's phenol reagent. After 5 min, 100 µL of 20% Na_2CO_3 were added and the mixture was allowed to stand for 10 min. Absorbance was read at 690 nm using a multi-well plate reader (Synergy 2, BioTek, Winooski, VT) and results expressed as mg gallic acid/g coproduct.

4.2.6. Measurement of tannin concentration

The method used to measure tannins was based on Bindon et al. (2014). Briefly, 50 µL methanol diluted and catechin standard or blank were added to each well followed by the addition of 200 µL of 8% acidified methanol and 50 µL of 1% vanillin (1:1) mixture until completing 200 µL. Fifty µL methanol and 200 µL of 4% acidified methanol were used as blank. Absorbance was read at 500 nm using a multi-well plate reader (Synergy 2, BioTek, Winooski, VT). The concentration of condensed tannins was calculated and expressed as mg catechin equivalents per g coproduct.

4.2.7. HPLC analysis

HPLC analysis of coproducts for anthocyanin profile was performed in triplicate using a Hitachi HPLC System (Hitachi High Technologies America, Inc., Schaumburg IL) equipped with a multi wavelength detector, L-7100 pump following a previously reported protocol with modifications (West and Mauer 2013). The injection volume was 20 µL. Flow rate was 400 µL/min and the gradient used was from 2% formic acid in water and 0 to 40% acetonitrile in a linear fashion using a Grace Prevail C18 HPLC column (5 µm, 250 × 4.6 mm, Columbia, MD) for 30 min. Peaks were identified based on the same retention time as available standards or

literature (West and Mauer 2013). The concentration of each anthocyanin was determined using a calibration curve with 5 points in the range of 50 to 2000 ng/mL of C3G standard. The calibration curve was obtained by plotting the area of cyanidin-3-glucoside peak versus its concentration. The calibration curve obtained gave a correlation coefficient (r^2) of 0.99.

4.2.8. Mass spectrometry

MS-MS analysis was performed on a Waters quadrupole time-of-flight (Q-Tof) Ultima mass spectrometer equipped with an electrospray ionization (ESI) interface and controlled by MassLynx V4.1 software (Waters Corp., Milford, MA). A Sunfire® C18 column (150 × 2.10 mm, 3.5 μm, Waters Corp., Milford, MA) was utilized for separation. The mobile phase was a linear gradient of 0.1% v/v formic acid (A) and acetonitrile (B) starting at 0% (B) and increasing to 40% (B) over 30 min; flow rate was 0.275 mL/min and the column was at ambient temperature. Waters default charge state recognition was used. Mass spectra was acquired in positive mode within the mass to charge ratio (m/z) range of 50 to 950 at cone voltages 35 V. Mass spectrometric data for anthocyanins were acquired in positive mode and monitored at 520 nm; phenolics were acquired at a negative mode and monitored at 280 nm (Collison et al. 2015). Anthocyanins and phenolics were identified based on the accurate mass measurements, tandem MS fragmentation and published literature.

4.2.9. Tristimulus colorimetric measurement

Color was measured using a Lovibond tintometer instrument (PFX990, The Tintometer Limited, Amesbury, England). The tintometer was calibrated using the following color scale parameters: CIELAB values L*, a* and b*; observer/illuminant: 10° and D65 and path length of 1 cm. Briefly, 2 to 3 mL extracts were acclimated at room temperature, placed in a disposable UV-Vis cuvette and color parameters L*, a* and b* measured and recorded. Color squares were

generated by converting L*, a* and b* values to R, G and B values using http://colormine.org/convert/rgb-to-lab and Microsoft powerpoint software. Hue angle, chroma, and saturation were calculated as follows: Hue angle = arctan (b*/a*); Chroma = sqrt (a^{*2} + b^{*2}); Saturation = Chroma/L*. The color difference (ΔE) was calculated according to CIE 1976 guideline as follows:

$$\Delta E^* = \sqrt{(L* - L_0)^2 + (a* - a_0)^2 + (b* - b_0)^2}$$

where L_0, a_0, and b_0 represent lightness, redness/greenness and blueness/yellowness of pericarp directly extracted with 2% formic acid (Sharma 2002).

4.2.10. Microscopy of colored corn

To soften kernels and facilitate sectioning, purple and blue kernels were soaked in deionized water for 12 h. Kernels were sectioned saggitally using a razor blade and embedded in 15 × 15 × 5 mm disposable vinyl specimen molds (Sakura Finetek USA, Inc., Torrance, CA) using Optimal Cutting Temperature (O.C.T.) compound (Thermo Fisher Scientific, Waltham MA). Embedded kernels were frozen and subsequently sectioned at 14 μm using a Leica Reichert Cryocut 1800 cryostat (Leica Biosystems, Buffalo Grove, IL) at -15°C. Bright field images of pericarp and aleurone layers were acquired using a Canon EOS Rebel T3i Digital SLR Camera (Canon U.S.A. Inc., Lake Success, NY) attached to an Olympus BX51 microscope (Olympus America Inc., Lombard, IL) at 40× magnification. This study was carried by Prof. Juvik's group in Department of Crop Sciences.

4.2.11. Statistical analysis

All analyses were conducted in at least three independent replicates. SAS version 9.4 software (SAS Institute, Cary, NY) was used. Differences among independent variables were determined using ANOVA by the proc GLM procedure and Tukey test ($p < 0.05$). Correlations

among different parameters were performed using Office Excel (Microsoft Corporation, Redmond, WA).

4.3. Results and Discussion

4.3.1. Anthocyanin concentrations of purple and blue corn coproducts

Wet milling fraction yields from purple and blue corn are summarized in Table 3.3. Starch yields for purple and blue corn were 63.4 and 61.5% (db), respectively. Dry milling fraction yields for purple and blue corn are summarized in Table 3.5. Large grits yield for blue corn was higher than purple corn, the values being 24.4 and 21.8% (db), respectively. For dry grind process, final ethanol concentrations for purple and blue corn were 14.5 and 14.3% (v/v), respectively and DDGS yields were 41.6 and 38.0% (db), respectively (Somavat et al. 2016).

MA in all coproducts from three conventional corn milling processes was measured to compare the distribution of anthocyanins after fractionation. For purple corn, MA in whole corn kernel after three sequential formic acid extractions were 4743 mg C3G equivalent per kg corn (db). Collison et al. (2015) showed MA concentration in purple corns varied from 1000 to 3000 C3G mg/kg (db) depending on varieties and growth environments. Yang et al. (2009) reported that four sequential extractions at optimized conditions yielded 5990 mg C3G/kg. MA concentrations observed in this study were comparable to those documented in above studies. However, the differences among anthocyanin contents reported in other studies can be explained partly due to different extraction methods employed, various purple corn cultivars used and different assays to measure anthocyanins.

The highest amount of anthocyanins in dry milling were observed in pericarp, which were 2175 mg C3G equivalent in 1 kg of corn and 26.8 g C3G equivalent per kg pericarp followed by small grits, large grits, germ and fines (Table 4.1). Pericarp accounted for 45.9% of

MA quantified in purple corn. In wet milling, maximum anthocyanins were quantified in steepwater (82.3%), the amount being 3903 mg C3G equivalent per kg purple corn. Total gluten slurry for the analysis constituted of gluten slurry filtrate and gluten, which were separated using Grade 1 Whatman® filter paper and a ceramic crucible and independently analyzed. Anthocyanins quantified in total gluten slurry were 833 mg C3G equivalent per kg purple corn, which was 17.6% of MA quantified in whole purple corn kernel. Dry grind coproduct DDGS contained only 28.7% of anthocyanins quantified in whole corn, which can be explained by deterioration of anthocyanins due to temperature and pH conditions experienced during the dry grind process (Wang et al. 2016).

MA concentration in whole blue corn from three sequential extractions was 391 mg C3G equivalent per kg corn (db) (Table 4.2). MA in blue corn with different growth environments and genetic backgrounds varied from 100 to 500 mg C3G equivalent per kg corn (db) (Collison et al. 2015). Abdel-Aal et al. (2006) reported MA in blue corn to be 200 mg C3G/kg corn; our findings are comparable to both reports. We found blue corn coproducts with the highest MA were different from purple corn coproducts. In dry milled blue corn, small grits contained the highest anthocyanins (108 mg C3G equivalent per kg corn) followed by pericarp, large grits, fines and germ. Small grits accounted for 27.6% of total anthocyanins while pericarp accounted for another 16.1%. Among wet milling coproducts, total gluten slurry had the highest anthocyanin concentration (255 mg C3G equivalent per kg corn) compared to other coproducts. Total gluten slurry contained 65.2% of total MA. Overall, anthocyanin concentration in blue corn was 8.2% of purple corn.

For purple corn, total anthocyanins quantified in dry milling and wet milling was 96 and 104%, respectively, of the total quantified in whole kernel. For blue corn, total anthocyanins

TABLE 4.1. Concentration of monomeric anthocyanins in purple corn dry milling, wet milling and dry grind coproducts*.

Process	Coproduct	Anthocyanins (ANC) (mg/kg of whole corn)[3]				ANC[#] (g/kg fraction)	Relative ANC[#] (Coproduct/Whole corn % db)
		First extraction	Second extraction	Third extraction	Sum of sequential extractions	Sum of sequential extractions	
Dry milling	Pericarp	1477 ± 84a**	395 ± 13b	303 ± 23c	2175 ± 121b***	26.8 ± 0.1a***	45.9
	Small grits	745 ± 123a	142 ± 23b	67 ± 6b	953 ± 152d	5.2 ± 1.0b	20.1
	Large grits	695 ± 95a	87 ± 13b	43 ± 9b	825 ± 117d	4.6 ± 0.5b	17.4
	Fines	350 ± 24a	48 ± 8b	23 ± 4c	422 ± 36e	1.4 ± 0.1de	8.9
	Germ	115 ± 13a	37 ± 4b	28 ± 4b	180 ± 20e	2.4 ± 0.1cd	3.8
Wet milling	Steepwater	-[2]	-	-	3903 ± 58a	-	82.3
	Gluten slurry[1]	-	-	-	833 ± 20d	-	17.6
	Starch	69.9 ± 15.9a	8.5 ± 3.1b	0.0b	78 ± 14e	0.1 ± 0.1e	1.7
	Fiber	26.8 ± 9.4a	15.5 ± 7.4a	24.9 ± 3.5a	67 ± 3e	0.6 ± 0.1de	1.4
	Germ	22.5 ± 5.3a	6.2 ± 0.7b	5.3 ± 2.4b	34 ± 5e	0.6 ± 0.1de	0.7
Dry grind	DDGS	979 ± 9a	199 ± 84b	183 ± 46b	1361 ± 139c	4.0 ± 0.4bc	28.7
	Whole corn	3610 ± 35a	851 ± 8b	282 ± 13c	4743 ± 55a	4.7 ± 0.1b	100

[1]Gluten slurry is composed of gluten and gluten slurry filtrate. Total anthocyanins equals sum of anthocyanins in both.
[2]No extraction was needed for liquid samples. Therefore, it was shown as "-" in the table. [#]Anthocyanins.
*These values represent Mean ± SD of three individual extractions of each sample.
[3]One-way ANOVA was conducted among three sequential extractions for the same fraction. One-way ANOVA was conducted among all fractions from the same processing for the sum of monomeric anthocyanins from sequential extractions. Same letters represent no significant ($p < 0.05$) difference, **within same row in a box for each process and ***within the column for all coproducts from three processes.

TABLE 4.2. Concentration of monomeric anthocyanins in blue corn dry milling, wet milling and dry grind coproducts*.

Process	Coproduct	Anthocyanins (ANC) (mg/kg of whole corn)[3]				ANC[#] (mg/kg coproduct)	Relative ANC[#] (Coproduct/Whole corn % db)
		First extraction	Second extraction	Third extraction	Sum of sequential extractions	Sum of sequential extractions	
Dry milling	Small grits	76.9 ± 0.6a**	19.6 ± 0.5b	11.6 ± 0.5c	108 ± 1.6c***	532 ± 6.5ab***	27.6
	Pericarp	36.4 ± 7.0a	14.9 ± 0.4a	11.6 ± 5.2a	63 ± 12.5cde	601 ± 70.4a	16.1
	Large grits	42.5 ± 5.0a	7.5 ± 0.6b	9.0 ± 0.1b	59 ± 5.6cdef	292 ± 18.1bc	15.1
	Fines	40.1 ± 3.1a	5.2 ± 0.5b	3.2 ± 2.0b	48 ± 5.6def	205 ± 14.2c	12.4
	Germ	10.5 ± 1.7a	4.4 ± 0.4b	1.6 ± 0.6b	16 ± 2.5f	210 ± 9.5c	4.2
Wet milling	Gluten slurry[1]	-[2]	-	-	255 ± 25.8b	-	65.2
	Steepwater	-	-	-	55 ± 0.1def	-	14.1
	Starch	20.3 ± 2.6a	7.6 ± 2..5b	0.0b	28 ± 5.1ef	54 ± 12.5c	7.1
	Germ	8.3 ± 1.3a	4.0 ± 1.0a	2.9 ± 0.2a	15 ± 3.1f	220 ± 19.6c	3.8
	Fiber	5.3 ± 0.9a	2.7 ± 0.01a	2.9 ± 0.2a	11 ± 1.1f	121 ± 7.4c	2.8
Dry grind	DDGS	55.6 ± 1.4a	25.2 ± 3.8b	5.8 ± 1.5c	87 ± 6.7cd	268 ± 10.3bc	22.1
	Whole corn	258 ± 10.9a	65 ± 6.4b	68 ± 3.5b	391 ± 20.8a	391 ± 20.8abc	100

[1]Gluten slurry is composed of gluten and gluten slurry filtrate. Total anthocyanins equals sum of anthocyanins in both.
[2]No extraction was needed for liquid samples. Therefore, it was shown as "-" in the table. [#]Anthocyanins.
*These values represent Mean ± SD of three individual extractions of each sample.
[3]One-way ANOVA was conducted among three sequential extractions for the same fraction. One-way ANOVA was conducted among all fractions from all processing for the sum of monomeric anthocyanins from sequential extractions. Same letters represent no significant ($p < 0.05$) difference, **within same row in a box for each process and ***within the column for all coproducts from three processes.

quantified in dry milling and wet milling was 75 and 93%, respectively, of the total quantified in the whole kernel. The wet milling process resulted in greater extraction of anthocyanins as compared to dry milling and dry grind processes. This was attributed to steeping corn in SO_2 and lactic acid. Application of sulfured water to black currants resulted in increased extraction of anthocyanins and other phenolics (Cacace and Mazza 2002).

4.3.2. Polyphenol and tannin concentrations in purple and blue corn coproducts

Phenolic compounds, with more than 8000 structural variations, contain one or more aromatic rings as well as hydroxyl groups and possess anti-carcinogenic, anti-mutagenic, anti-atherosclerotic and anti-inflammatory properties (Huang et al. 2010). As in the case of anthocyanins, maximum polyphenol concentrations in dry milled purple corn fractions were found in pericarp and in steepwater from wet milling corpoducts, the concentrations being 3.51 and 5.73 g gallic acid equivalent (GAE)/kg corn, respectively (Table 4.3). In blue corn dry milling coproducts, maximum polyphenol concentration was in pericarp and small grits, the amounts being 0.21 and 0.22 g GAE/kg corn, respectively. There were no differences between polyphenol concentration of blue corn pericarp and small grits. Gluten slurry contained the highest polyphenol concentration (1.28 g GAE/kg corn) for blue corn among wet milling coproducts. Polyphenols quantified in DDGS were 4.08 and 0.65 g GAE/kg corn for purple and blue corn, respectively.

These results are in agreement with other works. In a study by Montilla et al. (2011), the concentration of phenolic compounds in nine Bolivian purple corn cultivars ranged from 3.1 to 8.2 g GAE/kg. Urias-Lugo et al. (2015) reported the concentration of free phenolic compounds in five blue corn hybrids varied from 1.0 to 1.4 g GAE/kg, which was higher than our observations in this study. Overall polyphenol concentration of purple corn was six times higher

than blue corn. Comparing Table 4.1 and Table 4.3, anthocyanins accounted for more than 50% of polyphenols for most processing coproducts of purple corn. For example, polyphenols from purple corn pericarp were 26.8 g C3G/kg coproduct and polyphenols from purple corn pericarp were 43.3 g GAE/kg coproduct. However, MA concentrations of most coproducts from blue corn were less than half the polyphenol concentrations of the same coproduct (Table 4.2 and Table 4.3).

Tannins stabilize color by condensation reactions or copigmentation reactions with anthocyanins (Bautista-Ortín et al. 2005). For purple corn, pericarp and steepwater contained the majority of anthocyanins and also contained the highest tannin concentrations, which were 36.3 and 89.0 g catechin equivalent/kg corn, respectively as compared to the other coproducts (Table 4.4). For blue corn, unlike purple corn, small grits from dry milling and gluten slurry from wet milling contained the majority of tannins as compared to other coproducts.

4.3.3. Comparison of color intensities of purple and blue corn coproducts

Color parameters of all fractionation coproducts from purple and blue corn are displayed in Table 4.5 and 4.6, respectively. Chroma is an indicator of colorfulness. Chroma of whole purple corn extract was 90.5, much higher than chroma of blue corn (18.5), which was correlated with high concentration of anthocyanins in purple corn (r = x). Overall, purple corn formic acid extracts showed lower L*, higher a*, b* and chroma than the same coproducts in blue corn, indicating higher color intensities for purple corn coproducts than those from blue corn. Gluten slurry from both purple corn and blue corn were colorless because their pH was close to 5. After adjusting pH to 2.1, which is close to other formic acid extracts, the colorfulness of gluten slurry was displayed. For purple corn, the color intensity of pericarp extract was greatest in dry milling while for wet milling, the steepwater was greatest as displayed in color squares. However, for

TABLE 4.3. Concentration of polyphenols in dry milling, wet milling and dry grind coproducts.

		Purple corn		Blue corn	
Process	**Coproduct**	**g gallic acid equivalent/kg coproduct**	**g gallic acid equivalent/kg corn**	**g gallic acid equivalent/kg coproduct**	**g gallic acid equivalent/kg corn**
Dry milling	Pericarp	43.3 ± 0.3a	3.5 ± 0.1c	2.0 ± 0.25a	0.21 ± 0.02d
	Small grits	6.5 ± 0.8c	1.2 ± 0.1e	1.1 ± 0.01b	0.22 ± 0.01d
	Large grits	4.3 ± 0.3d	0.8 ± 0.1f	0.7 ± 0.03b	0.15 ± 0.01d
	Fines	2.0 ± 0.1ef	0.6 ± 0.1f	0.7 ± 0.04bc	0.15 ± 0.01d
	Germ	3.7 ± 0.2de	0.3 ± 0.1g	1.0 ± 0.01b	0.08 ± 0.01d
Wet milling	Steepwater	-	5.73 ± 0.03a	-	0.89 ± 0.06b
	Gluten slurry[1]	-	2.36 ± 0.04d	-	1.28 ± 0.04a
	Fiber	2.41 ± 0.13e	0.26 ± 0.05g	1.01 ± 0.02b	0.07 ± 0.01d
	Starch	0.47 ± 0.01f	0.25 ± 0.00g	0.83 ± 0.01c	0.10 ± 0.01d
	Germ	2.04 ± 0.22ef	0.11 ± 0.01g	0.20 ± 0.02b	0.07 ± 0.01d
Dry grind	DDGS	11.31 ± 0.15b	4.08 ± 0.02b	2.04 ± 0.10a	0.65 ± 0.04c

Values represent polyphenols quantified in the first extraction from each coproduct.
[1]Gluten slurry is composed of gluten and gluten slurry filtrate. Total polyphenols equals sum of polyphenols in both.
Unavailable data was shown as "-"
These values represent Mean ± SD of three individual extractions of each sample.
Same letters represent no significant ($p < 0.05$) difference between coproducts from any process.

TABLE 4.4. Concentration of tannins in dry milling, wet milling and dry grind coproducts.

		Purple corn		Blue corn	
Process	**Coproduct**	**g catechin equivalent/kg coproduct**	**g catechin equivalent/kg corn**	**g catechin equivalent/kg coproduct**	**g catechin equivalent/kg corn**
Dry milling	Pericarp	447 ± 60.9a	36.3 ± 6.3b	6.6 ± 2.2bc	0.7 ± 0.2de
	Small grits	72 ± 9.8b	13.1 ± 2.1c	11.4 ± 0.7b	2.3 ± 0.1bc
	Large grits	48 ± 5.6b	8.7 ± 1.3cd	6.5 ± 0.4bc	1.3 ± 0.1cde
	Fines	22 ± 1.4b	6.5 ± 0.1cd	5.7 ± 0.1bc	1.3 ± 0.1cde
	Germ	28 ± 1.4b	2.1 ± 0.1cd	4.3 ± 0.2bc	0.3 ± 0.1e
Wet milling	Steepwater	-	89.0 ± 0.7a	-	1.8 ± 0.1bcd
	Gluten slurry[1]	-	7.7 ± 0.6cd	-	6.2 ± 0.5a
	Fiber	5.1 ± 0.4b	0.5 ± 0.1d	3.3 ± 0.3c	0.3 ± 0.1e
	Starch	1.8 ± 0.3b	1.0 ± 0.2d	0.9 ± 0.2c	0.5 ± 0.1e
	Germ	6.3 ± 1.3b	0.3 ± 0.1d	3.1 ± 0.1c	0.2 ± 0.1e
Dry grind	DDGS	73 ± 3.1b	26 ± 0.7b	8.6 ± 1.8a	2.7 ± 0.5b

Values represent tannin concentration in the first extraction from each coproduct.
[1]Gluten slurry is composed of gluten and gluten slurry filtrate. Total tannins equals sum of tannins in both.
Unavailable data was shown as "-".
These values represent Mean ± SD of three individual extractions of each sample.
Same letters represent no significant ($p < 0.05$) difference between coproducts from any process.

blue corn, the color squares for small grits from dry milling and gluten slurry from wet milling had greatest intensity.

4.3.4. Profile of anthocyanins and phenolics in purple and blue corn coproducts

Anthocyanin composition of all purple and blue corn coproducts were determined by HPLC at 520 nm. For purple corn fractions, the highest concentration of condensed form was found in whole corn and in steepwater, the values being 269 and 260 mg/kg dry corn, respectively (Table 4.7). Condensed forms are heterodimers consisting of an anthocyanin and a flavonol, typically catechin or epicatechin (Li et al. 2017). C3G concentration was highest in whole corn (1135 mg/kg corn). Anthocyanin concentration was highest in steepwater from the wet milling process, followed by whole purple corn kernel and then in the pericarp recovered from dry milled corn, which was in agreement with the MA concentations shown in Table 4.1 and Fig. 4.1. Among acylated anthocyanins, concentration of cyanidin 3-O-(6"-malonyl-glucoside) was higher than pelargonidin 3-O-(6"-malonyl-glucoside) and peonidin 3-O-(6"-malonyl-glucoside) for all fractions.

For blue corn fractions, instead of whole corn, condensed form was the highest in pericarp (1.9 mg/kg corn) and C3G concentration was highest in gluten slurry (10.1 mg/kg corn). Unlike in purple corn coproducts, highest concentration of anthocyanins was found in gluten slurry instead of steepwater (Table 4.7). Cyanidin 3-O-(6"-malonyl-glucoside) was the dominant form among anthocyanins in all fractions. The concentrations of condensed form, namely perlargonidin-3-glucoside and peonidin-3-glucoside were too low to be detected by the HPLC for most of the fractions. Phenolic compound profile of purple corn (HPLC 280 nm, Fig. 4.2) was similar to anthocyanin profile (HPLC 520 nm), because the majority of phenolic compounds in purple corn are anthocyanins. Interestingly, in all blue corn coproducts, the

TABLE 4.5. Color parameters of dry milling, wet milling and dry grind coproducts from purple corn.

Process	Coproduct	pH	L*	a*	b*	Hue Angle	Chroma	Δ E	Color Square
Dry milling	Pericarp	2.1 ± 0.02	20.1 ± 0.1	49.0 ± 0.3	27.6 ± 0.3	29.4 ± 0.1b	56.3 ± 0.4c	45.3 ± 0.4g	
	Large grits	2.0 ± 0.01	83.5 ± 0.1	18.4 ± 0.7	4.5 ± 0.2	13.9 ± 1.2e	18.9 ± 0.6gh	83.8 ± 0.2bc	
	Small grits	2.1 ± 0.02	76.3 ± 0.5	29.2 ± 0.1	8.8 ± 0.5	16.8 ± 0.9de	30.5 ± 0.2ef	71.7 ± 0.6de	
	Germ	2.2 ± 0.03	90.1 ± 0.1	11.6 ± 0.6	2.4 ± 0.1	11.6 ± 0.4e	11.9 ± 0.6ghi	92.3 ± 0.4ab	
	Fines	2.1 ± 0.01	82.5 ± 0.3	15.8 ± 0.7	6.5 ± 0.5	22.3 ± 2.4cd	17.1 ± 0.5ghi	83.6 ± 0.1bc	
Wet milling	Germ	2.1 ± 0.01	84.2 ± 2.4	21.2 ± 4.0	5.3 ± 0.9	14.0 ± 0.2e	21.9 ± 4.1g	82.1 ± 4.0c	
	Fiber	2.1 ± 0.01	79.0 ± 0.4	23.7 ± 2.7	7.3 ± 0.1	17.4 ± 1.9de	24.9 ± 2.6fg	76.9 ± 1.7cd	
	Gluten	2.1 ± 0.01	57.9 ± 0.7	55.8 ± 2.0	24.3 ± 1.1	23.6 ± 0.1bc	60.9 ± 2.3bc	41.0 ± 1.6g	
	Starch	2.1 ± 0.01	91.7 ± 0.4	9.4 ± 0.6	2.5 ± 0.1	14.9 ± 0.2e	9.8 ± 0.6ij	94.3 ± 0.7a	
	Steepwater	4.1 ± 0.01	28.1 ± 0.6	53.0 ± 0.1	40.4 ± 0.5	37.4 ± 0.3a	66.6 ± 0.3b	29.9 ± 0.7h	
	Steepwater[1]	2.1	15.5 ± 0.3	42.6 ± 0.4	20.0 ± 0.4	25.2 ± 0.3bc	47.1 ± 0.5d	55.9 ± 0.6f	
	Gluten slurry	4.7 ± 0.1	85.9 ± 1.7	7.2 ± 0.5	3.9 ± 0.7	28.4 ± 2.5b	8.2 ± 0.7g	92.1 ± 1.5ab	
	Gluten slurry[1]	2.1	66.2 ± 7.1	35.4 ± 1.8	10.7 ± 1.7	16.6 ± 1.6de	37.0 ± 2.2e	63.5 ± 4.6ef	
Dry grind	DDGS	2.2 ± 0.01	42.7 ± 0.4	64.4 ± 0.1	52.4 ± 0.1	39.1 ± 0.1a	83.0 ± 0.1a	9.7 ± 0.1i	
Whole kernel		1.9 ± 0.01	44.7 ± 0.3	65.7 ± 0.2	61.9 ± 0.1	43.2 ± 0.1a	90.5 ± 0.1a	-	

All the color and pH measurements were based on the first extraction of each coproduct.

[1]pH of steepwater and gluten slurry were adjusted to 2.13 to compare the color difference between their original pH and lower pH close to other coproducts.

Δ E was the color difference between each coproduct and whole kernel

One-way ANOVA was conducted across the column.

Gluten slurry is composed of gluten and gluten slurry filtrate. Gluten slurry filtrate was obtained by filtration of gluten slurry to remove particles.

Unavailable data was shown as "-". Means followed by the same letter in one column are not different.

TABLE 4.6. Color parameters of dry milling, wet milling and dry grind coproducts from blue corn.

Process	Coproduct	pH	L*	a*	b*	Hue Angle	Chroma	ΔE	Color Square
Dry milling	Pericarp	2.2 ± 0.01	79.3 ± 0.6	29.3 ± 1.3	6.6 ± 0.7	12.7 ± 6.2c	30.1 ± 1.0a	13.8 ± 0.2c	
	Large grits	2.2 ± 0.04	83.5 ± 0.1	18.4 ± 0.7	4.5 ± 0.2	13.0 ± 1.2c	18.9 ± 0.6b	3.7 ± 0.1f	
	Small grits	2.2 ± 0.02	76.3 ± 0.5	29.2 ± 0.1	8.8 ± 0.5	16.8 ± 0.9c	30.5 ± 0.2a	16.3 ± 0.5abc	
	Germ	2.3 ± 0.01	90.1 ± 0.1	11.6 ± 0.6	2.4 ± 0.1	11.6 ± 0.4c	11.9 ± 0.6cde	7.2 ± 0.6ef	
	Fines	2.1 ± 0.03	82.5 ± 0.3	15.8 ± 0.7	6.5 ± 0.5	22.3 ± 2.4c	17.1 ± 0.5bc	5.8 ± 0.8ef	
Wet milling	Germ	2.2 ± 0.01	89.7 ± 0.6	9.8 ± 1.3	3.3 ± 0.7	19.4 ± 6.2c	10.4 ± 1.0def	8.8 ± 1.0de	
	Fiber	2.2 ± 0.03	85.5 ± 1.8	5.4 ± 0.7	5.6 ± 0.6	46.0 ± 7.0b	7.8 ± 0.1efg	13.0 ± 1.0cd	
	Gluten	2.1 ± 0.02	75.8 ± 1.2	32.2 ± 1.3	8.4 ± 0.7	14.6 ± 0.5c	33.3 ± 1.5a	18.7 ± 1.3ab	
	Starch	2.1 ± 0.03	94.2 ± 0.2	4.2 ± 0.6	1.2 ± 0.1	15.8 ± 1.8c	4.3 ± 0.6gh	15.8 ± 0.6abc	
	Steepwater	4.1 ± 0.02	95.6 ± 0.1	-0.8 ± 0.1	3.9 ± 0.1	-78.4 ± 0.3d	4.0 ± 0.1gh	20.7 ± 0.1a	
	Steepwater[1]	2.2	92.8 ± 0.1	5.2 ± 0.1	3.6 ± 0.1	35.4 ± 0.6b	6.2 ± 0.1fgh	14.1 ± 0.1bc	
	Gluten Slurry	5.0 ± 0.01	95.6 ± 0.1	0.1 ± 0.1	1.2 ± 0.1	87.5 ± 0.1a	1.2 ± 0.1h	20.1 ± 0.1a	
	Gluten Slurry[1]	2.2	72.6 ± 0.6	27.9 ± 2.0	7.1 ± 0.7	14.3 ± 0.4c	28.8 ± 2.1a	19.1 ± 2.2a	
Dry grind	DDGS	2.2 ± 0.02	88.1 ± 0.9	12.8 ± 0.6	4.6 ± 0.4	19.8 ± 0.9c	13.6 ± 0.7cd	5.6 ± 0.6ef	
Whole kernel	Whole corn	2.2 ± 0.02	87.1 ± 0.1	18.1 ± 0.5	3.8 ± 0.1	11.8 ± 0.2	18.5 ± 0.5	-	

All the color and pH measurements were based on the first extraction of each coproduct.

[1]pH of steepwater and gluten slurry were adjusted to 2.13 to compare the color difference between their original pH and lower pH close to other coproducts.

Δ E was compared to the color of whole kernel

Gluten slurry is composed of gluten and gluten slurry filtrate. Gluten slurry filtrate was obtained by filtration of gluten slurry to remove particles.

Unavailable data was shown as "-". Means followed by the same letter in one column are not different.

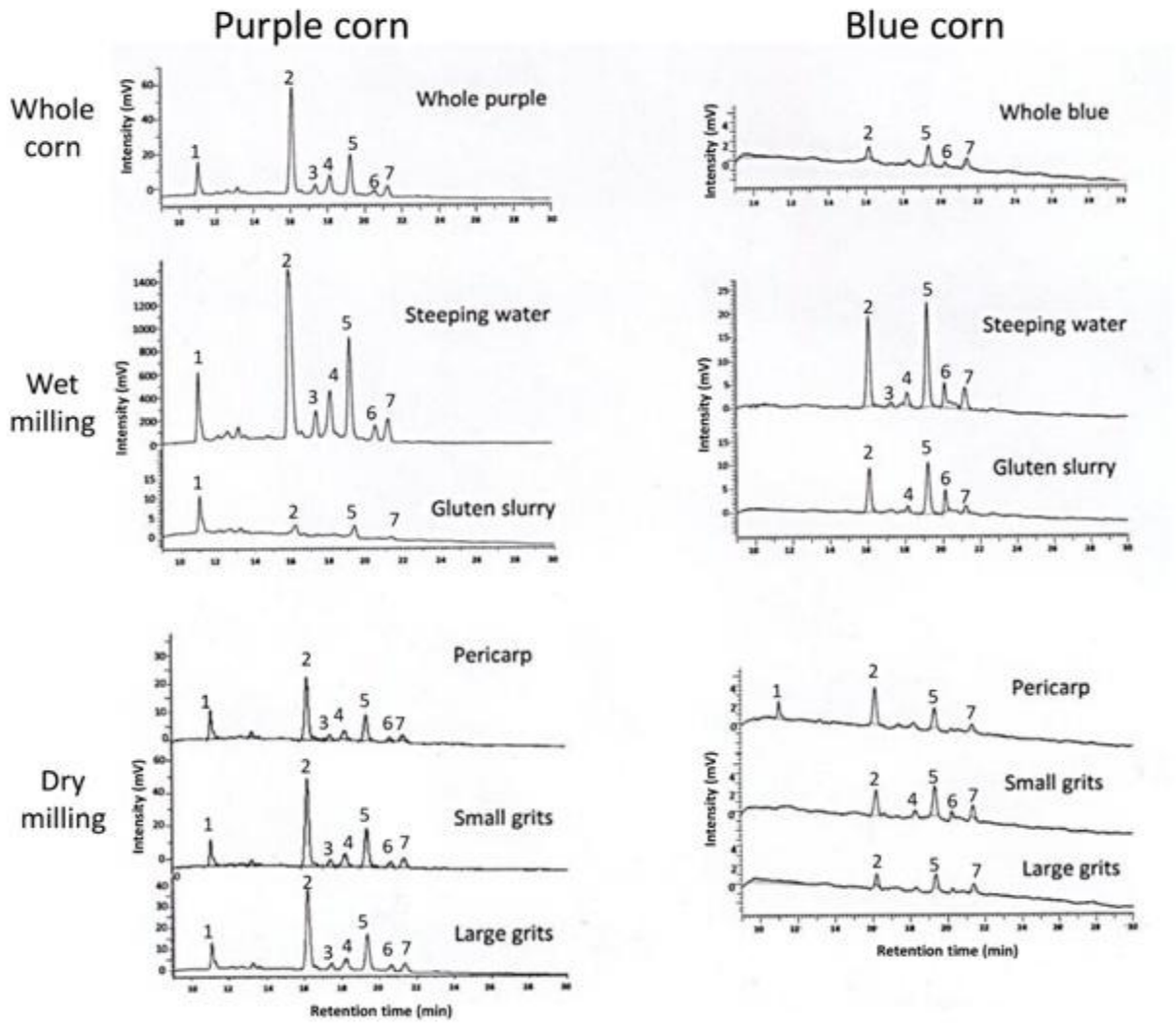
highest peak was at retention time of 21.6 min (HPLC 280 nm, Fig. 4.3), which was a compound containing cyanidin derivative according to the observations from LC/MS analysis (de Mejia et al. 2005). To the best of our knowledge, we are reporting for the first time, the distribution of anthocyanins and phenolic compounds in anthocyanin rich extracts of colored corns with different genotype and phenotype with respect to the location of pigments. Researchers have reported distribution of different phenolic or pigmented compounds in other grains/crops. The concentration of carotenoids in Indian corn was highest in germ, followed by aleurone and endosperm (Masisi et al. 2015). In another study (Ndolo and Beta 2014) involving composition and distribution of phenolic acids in cereal grains, the investigators reported that phenolic acid concentrations in pericarp and aleurone layer were higher than the concentrations present in germ and endosperm of yellow dent corn.

Overall, yellow dent corn contained the highest amount of phenolic acids as compared to wheat, barley and oats. Distribution of anthocyanins in wheat and barley also has been documented. Anthocyanins were concentrated 2.3 fold in grain fraction of blue wheat as compared to the whole grain (Abdel-Aal et al. 2008). In another work, the investigators reported that six times more anthocyanins were present in the bran rich fractions of yellow and purple barley as compared to the whole seed (Bellido and Beta 2009). Our observations are similar to these studies and higher concentrations of anthocyanins were recorded in the outer layers of corn, i.e. pericarp and aleurone. This study focused on the composition and distribution of anthocyanins in fractionation coproducts of colored corns and enhances the existing knowledge of the role of processing on concentrating anthocyanins into different coproduct streams.

TABLE 4.7. Anthocyanin profiles of coproducts from wet milling, dry milling and dry grind processes.

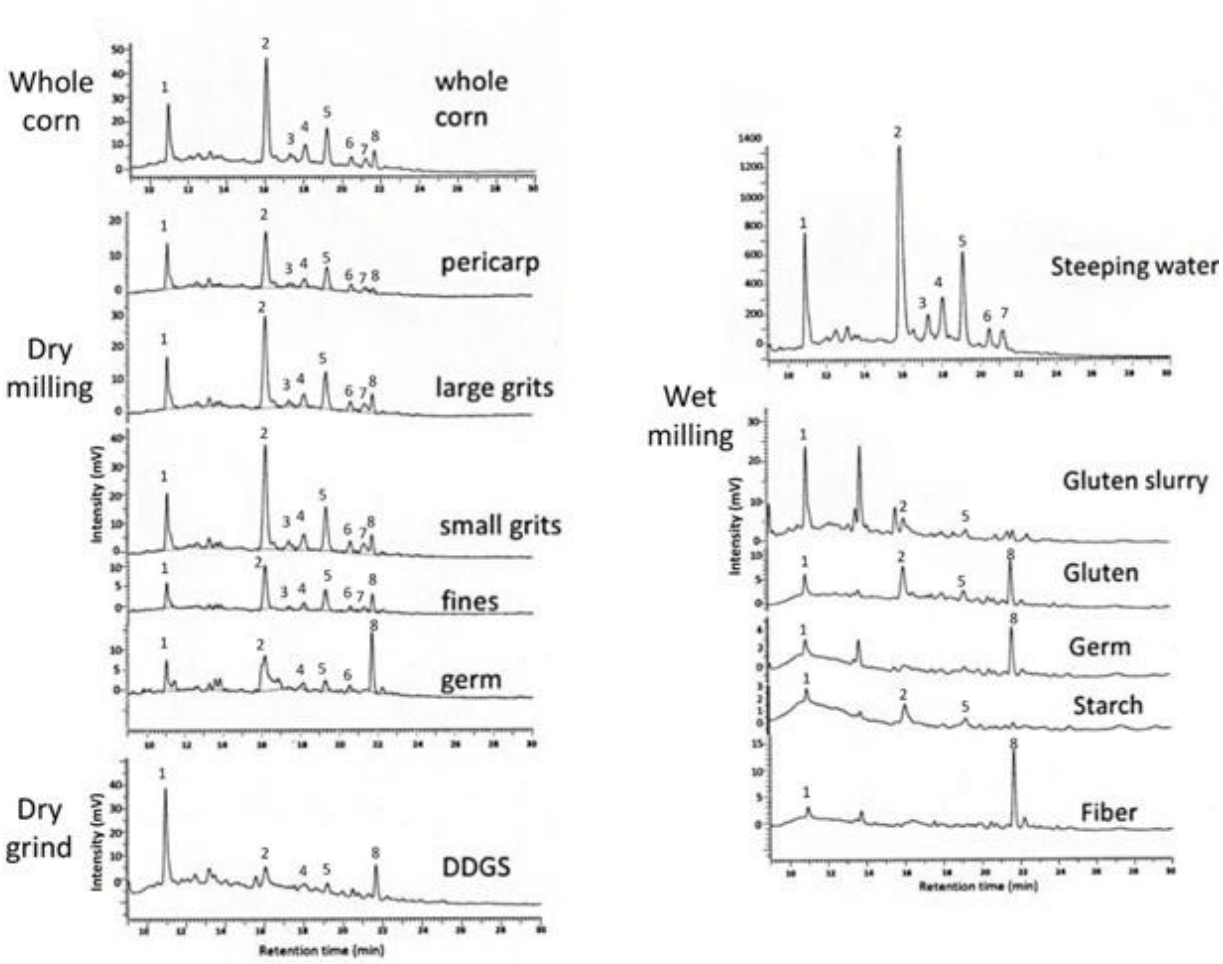
Anthocyanin concentration (mg/kg corn)												
			Wet milling				Dry milling					Dry grind
		Whole corn	Steepwater	Gluten slurry	Germ	Starch	Pericarp	Large grits	Small grits	Germ	Fines	DDGS
Purple corn	CF	269 ± 2.5a	260 ± 0.5a	25.2 ± 10.9cd	0.4 ± 0.1e	1.2 ± 0.1e	95.9 ± 1.1b	27.4 ± 0.8c	36.1 ± 0.3c	5.3 ± 0.3de	16.4 ± 0.4cde	109.3 ± 1.9b
	C3G	1135 ± 12.9a	1079 ± 0.5b	21.7 ± 1.4g	0.7 ± 0.1g	16.0 ± 1.1g	313.4 ± 4.7c	120.6 ± 2.8e	156.6 ± 3.4d	14.3 ± 1.1g	76.9 ± 2.1f	71.6 ± 2.6f
	Pr3G	116 ± 1.1b	198 ± 0.03a	1.2 ± 0.1f	n/d	n/d	28.4 ± 3.8c	9.3 ± 0.5e	15.4 ± 0.6d	1.6 ± 0.3f	4.7 ± 0.6ef	8.0 ± 0.2e
	Pn3G	285 ± 21.9b	406 ± 0.4a	2.8 ± 0.1d	n/d	0.5 ± 0.5d	55.4 ± 6.4c	28.8 ± 1.4cd	34.4 ± 3.2cd	4.0 ± 0.6d	11.5 ± 1.6d	11.3 ± 0.1d
	C36MG	398 ± 5.6b	495 ± 4.3a	13.3 ± 4.1f	0.6 ± 0.1f	5.2 ± 0.2f	128.4 ± 9.1c	56.7 ± 1.7d	69.9 ± 1.2d	5.5 ± 0.6f	37.3 ± 1.2e	35.0 ± 0.1e
	Pg36MG	52 ± 0.9b	105 ± 0.6a	1.2 ± 0.2fg	0.03 ± 0.1g	n/d	15.7 ± 0.1c	5.5 ± 0.3e	11.7 ± 1.2d	1.1 ± 0.1fg	4.2 ± 0.1ef	2.7 ± 0.8efg
	Pn36MG	145 ± 2.0a	135 ± 0.3b	1.9 ± 0.2e	n/d	n/d	34.5 ± 5.6c	16.4 ± 0.4d	22.2 ± 0.8d	1.8 ± 0.2e	5.8 ± 1.7e	2.9 ± 0.6e
Blue corn	CF	n/d	n/d	n/d	0.2 ± 0.2b	n/d	1.9 ± 0.1a	n/d	n/d	n/d	n/d	n/d
	C3G	17.4 ± 0.8b	10.1 ± 0.1c	37.5 ± 0.4a	1.0 ± 0.1f	2.8 ± 0.2ef	5.2 ± 0.3d	2.8 ± 0.1ef	8.2 ± 0.4c	0.7 ± 0.1f	3.6 ± 0.5de	4.1 ± 0.7de
	Pr3G	n/d	0.5 ± 0.1	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
	Pn3G	n/d	1.6 ± 0.1b	4.0 ± 0.5a	n/d	n/d	n/d	n/d	1.6 ± 0.3b	n/d	n/d	n/d
	C36MG	40.1 ± 6.5b	12.0 ± 0.1c	55.5 ± 0.7a	0.9 ± 0.1d	3.1 ± 2.1cd	3.7 ± 0.1cd	4.5 ± 0.5cd	12.1 ± 0.3c	0.7 ± 0.2d	5.4 ± 0.8cd	2.9 ± 0.4cd
	Pg36MG	6.5 ± 3.9b	3.6 ± 0.8b	23.4 ± 4.1a	n/d	n/d	n/d	n/d	1.5 ± 0.2b	n/d	n/d	1.4 ± 0.2b
	Pn36MG	7.4 ± 3.8a	3.6 ± 0.1a	7.1 ± 1.0a	n/d	n/d	1.1 ± 0.1a	2.2 ± 0.5a	3.8 ± 0.1a	n/d	2.1 ± 0.2a	n/d

n/d: non-detectable, concentration less than 0.05 mg/g fraction was shown as non-detectable.
One-way ANOVA was conducted for each anthocyanin among all the coproducts for purple corn or blue corn. Means followed by the same letter are not different.
CF: condensed form; C3G: cyanidin-3-glucoside; Pr3G: Perlargonidin-3-glucoside; Pn3G: Peonidin-3-glucoside; C36MG: Cyanidin 3-O-(6"-malonyl-glucoside); Pg36MG: Pelargonidin 3-O-(6"-malonyl-glucoside); Pn36MG: Peonidin 3-O-(6"-malonyl-glucoside).



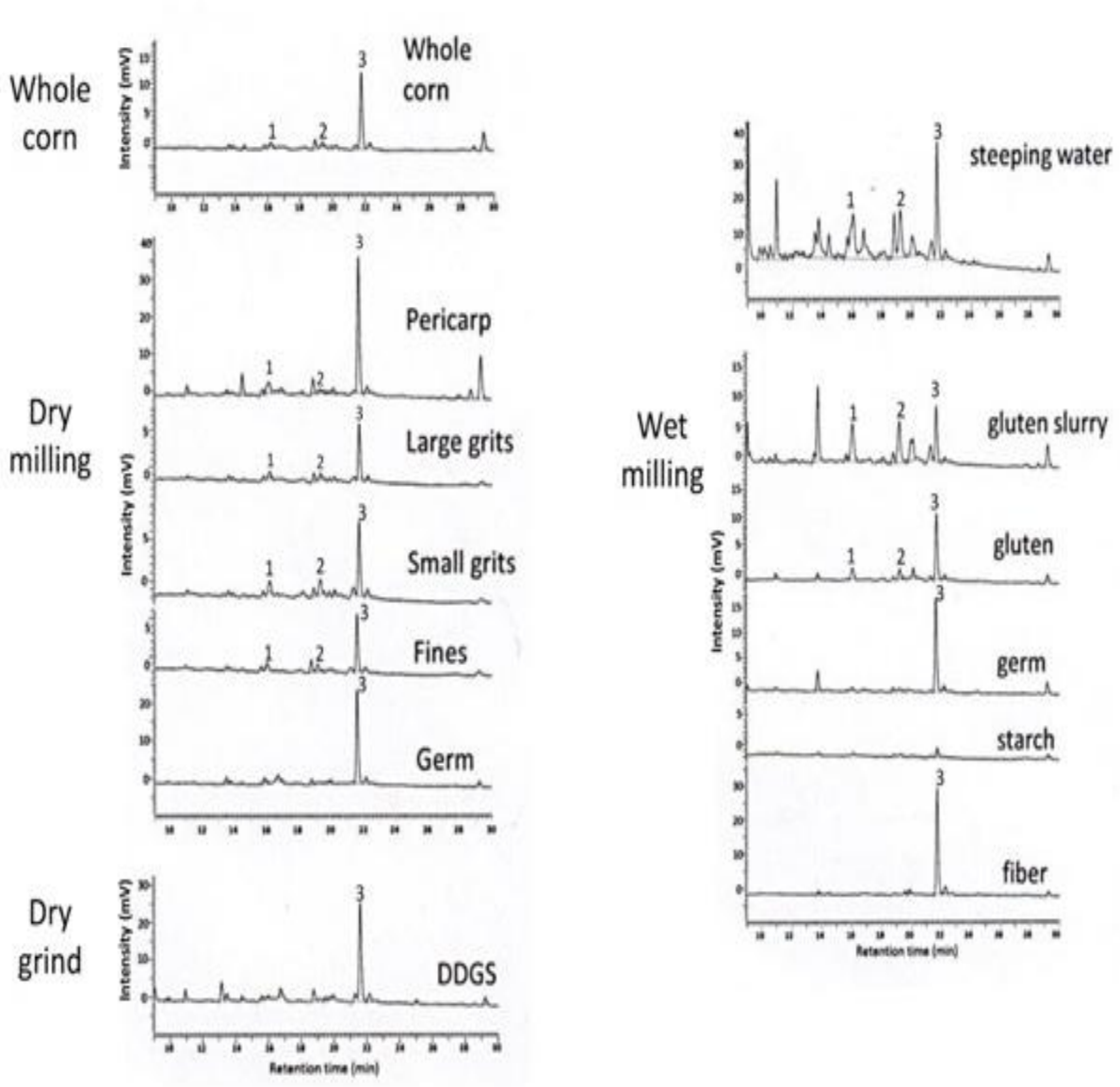
Peak	Retention time (min)	Identity
1	11.07	Condensed form
2	16.17	Cyanidin-3-glucoside
3	17.45	Pelargonidin-3-glucoside
4	18.19	Peonidin-3-glucoside
5	19.29	Cyanidin-3-O-(6”-malonyl-glucoside)
6	20.61	Pelargonidin-3-O-(6”-malonyl-glucoside)
7	21.31	Peonidin-3-O-(6”-malonyl-glucoside)

Fig. 4.1. HPLC chromatograms of acidified water extracts from purple and blue corn dry milling, wet milling fractions and whole corn at 520 nm and possible identities of main peaks by LC/MS.



Peak	Retention time (min)	Identity
1	11.07	Condensed form
2	16.17	Cyanidin-3-glucoside
3	17.45	Pelargonidin-3-glucoside
4	18.19	Peonidin-3-glucoside
5	19.29	Cyanidin-3-O-(6”-malonyl-glucoside)
6	20.61	Pelargonidin-3-O-(6”-malonyl-glucoside)
7	21.31	Peonidin-3-O-(6”-malonyl-glucoside)
8	21.77	Cyanidin derivative

Fig. 4.2. HPLC chromatograms of purple corn acidified water extracts from dry milling, wet milling and dry grind fractions at 280 nm and possible identities of main peaks by LC/MS.



Peak	Retention time (min)	Identity
1	16.17	Cyanidin-3-glucoside
2	19.29	Cyanidin-3-O-(6”-malonyl-glucoside)
3	21.77	Cyanidin derivative

Fig. 4.3. HPLC chromatograms of blue corn acidified water extracts from dry milling, wet milling and dry grind fractions at 280 nm and possible identities of main peaks by LC/MS.

4.3.5. Bright field images of purple and blue corn kernel sections

Microscopic images of sagittal sections of purple and blue corn at 40× magnification are shown in Fig. 4.4. In purple corn, pericarp was thick and was dark red in color. However, aleurone, the single cuboidal layer between the pericarp and endosperm, was transparent. In contrast to purple corn, color in blue kernels was restricted to the aleurone layer; the pericarp layer was thin and transparent. Small grits and gluten slurry were coproducts that contained the highest MA in blue corn after dry milling and wet milling processes, respectively. This may be attributed to most anthocyanins in blue corn being concentrated in the aleurone layer instead of pericarp as in purple corn.

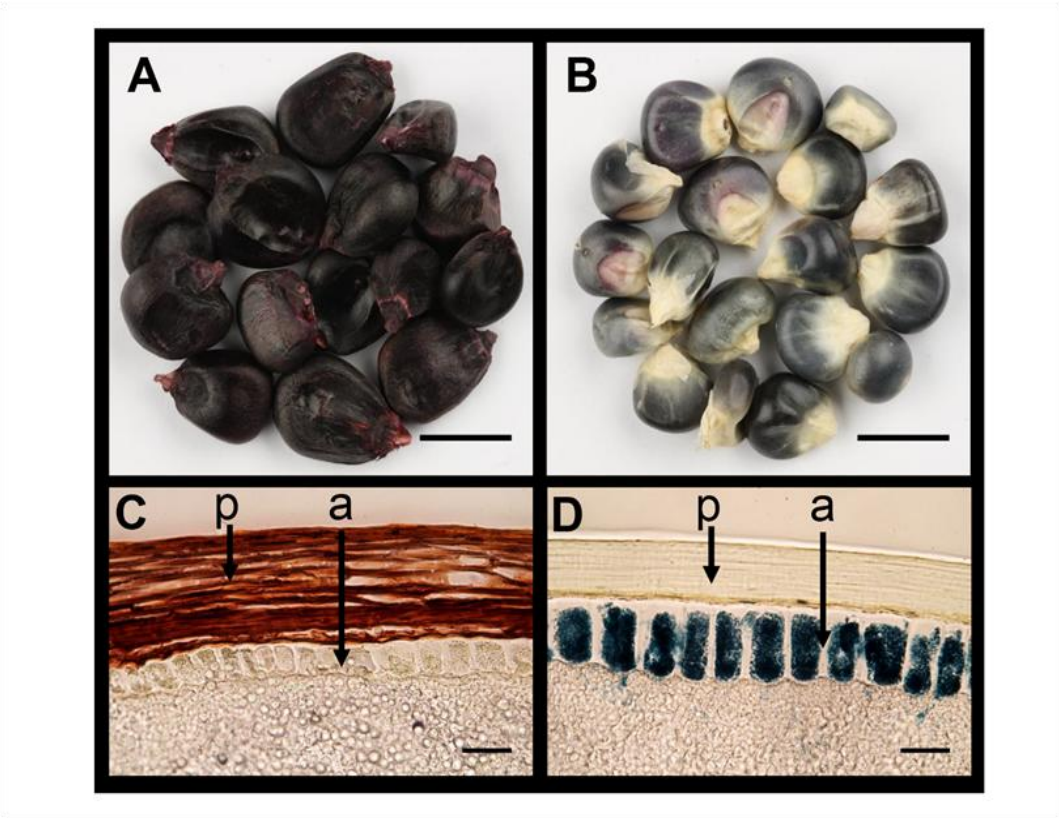


Fig. 4.4. Morphological differences between kernels of purple (A) and blue (B) corns; bright field micrographs taken at 40× magnification show pigment accumulation in the pericarp tissue (p) of purple corn (C) and the aleurone tissue (a) of blue corn (D). (Scale bars correspond to 1 cm in A and B and 50 µm in C and D). Image courtesy: Ms. Laura Chatham (Crop Sciences).

4.4. Conclusions

Maximum anthocyanins were concentrated either in pericarp or aleurone layer depending upon the corn cultivar. For purple corn, maximum anthocyanin concentration was in the pericarp separated from the kernel using dry milling process. Maximum blue corn anthocyanins were concentrated in the aleurone layer and were extracted in the gluten slurry, a coproduct of corn wet milling. Therefore, the optimal process flows for recovering anthocyanins from colored corn cultivars depend upon the physical distribution of anthocyanins in that particular corn. Corn processing can generate coproducts which have higher concentrations of anthocyanins based on the corn phenotype. Localization of anthocyanins in corn kernel provides a unique processing advantage for colored corns over other fruits and vegetables for anthocyanin extraction. Corn fractionation processes can be tailored so as to extract maximum anthocyanins with improved process economics while at the same time remaining fractionation coproducts, which remain unaffected, can be utilized facilitating economic anthocyanin recovery. We present a better understanding of anthocyanin distribution in different color corn coproducts from various milling processes, paving the way for their economical extraction.

Chapter 5. Anthocyanin recovery from purple corn pericarp using wet milling chemicals

5.1. Introduction

Research regarding the distribution and yield of anthocyanins in coproducts of different milling processes, has led to three important insights. The location of pigments inside the corn kernel depends upon corn genetics. In the case of purple corn, the maximum amount of anthocyanins was present in pericarp; whereas, for blue corn the maximum amount was concentrated in aleurone layer. Steepwater, a coproduct of corn wet milling, contained the maximum amount of anthocyanins among the coproducts from the three processes. Purple corn contained 12 times more anthocyanins as compared to blue corn. Based on these observations, it was determined that the most efficient process for anthocyanin recovery will involve purple corn. Purple corn pericarp, containing the maximum anthocyanins, can be recovered at the front end by using dry milling. The remaining corn endosperm can be processed further by using conventional techniques. Recovered corn pericarp, which is 8 to 10% of the starting material, can be processed selectively for anthocyanin recovery, yielding economic advantages.

During wet milling, corn is steeped in water containing 0.20% sulfur dioxide (SO_2) generated from sodium metabisulfite ($Na_2S_2O_5$), often used in lab studies but not always commercially, and 0.50% lactic acid (L.A.) at 50 to 52°C. L.A. helps to soften the corn kernel and facilitates faster SO_2 sorption, which results in higher starch yields (Eckhoff et al. 1993; Roushdi et al. 1981). Inside the corn kernel, SO_2 attacks the disulfide bonds of the protein matrix encapsulating the starch granules. Once the endosperm protein matrix is dispersed, the starch granules become free, and the overall starch recovery is increased (Watson 1984; Watson and Sanders 1961). In the food industry, especially in wine making, SO_2 is used to control

undesirable microorganisms, to prevent browning and to serve as an antioxidant (Santos et al. 2012). Because of the high solubility of anthocyanins in water, an extraction with water can be used to obtain a high yield of anthocyanins from corn (Jing and Giusti 2007). Investigators have reported the addition of SO_2 during processing facilitates greater extraction of pigments (Cacace and Mazza 2002; Dallas and Laureano 1994; Lee et al. 2005; Lee and Wrolstad 2004). However, the use of SO_2 is an important health and environmental concern due to its volatility and reactivity (Santos et al. 2012). Yang et al. (2005) proposed the use of sulfite salts in corn wet milling to reduce SO_2 emissions to the atmosphere.

Based on insights from the preliminary processing of purple corn, the current study was designed to increase anthocyanin extraction from purple corn pericarp by using various combinations of chemicals commonly used in corn wet milling, namely, SO_2 (generated from added $Na_2S_2O_5$) and L.A. The effects of steeping chemicals on anthocyanin extract profiles were investigated.

5.2. Materials and Methods

5.2.1. Materials

Commercially available purple corn cultivar (*Zea mays L.*) was purchased from an online vendor (Lot No. L670106, Angelina's Gourmet, Swanson, CT). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

5.2.2. Pericarp recovery and steeping

Purple corn was dry milled and pigment-rich pericarp was recovered by using 1 kg scale procedure developed by Rausch et al. (2009). Pericarp (10 g) was steeped using four combinations of steeping chemicals. The treatments were a) pericarp steeped in 125 mL of

deionized water, b) pericarp steeped in 125 mL of deionized water and 0.27 g of sodium metabisulfite (SO_2), c) pericarp steeped in 125 mL of deionized water and 0.58 mL of 85% L.A. and d) pericarp steeped in 125 mL of deionized water, 0.27 g of SO_2 and 0.58 mL of 85% L.A.

These four treatments were incubated at 52°C for 24 hr and stirred at 100 rpm, simulating a lab scale steeping procedure for the wet milling of corn. Our specific aim was to determine the optimal anthocyanin recovery combination and to determine its effects on the resultant color. After steeping, the liquid was separated from the pericarp by using a ceramic crucible. The remaining pericarp was dried at room temperature and further analyzed to measure the remaining anthocyanins. All treatments were performed three times.

5.2.3. Extraction of anthocyanins from untreated pericarp

Dry untreated pericarp was ground using a Kitchen-Aid coffee grinder for 25 sec. Ground material was passed through a 35 mesh sieve and material that did not pass through the sieve was ground again for another 25 sec and passed through the same sieve. Materials passing through the sieve were combined and used for extraction. Ground material (0.5 g) was suspended in 20 mL (40:1 liquid-to-solid ratio) of 2% aqueous formic acid and mixed for 2 hr at room temperature. The suspension was filtered and the resulting filtrate was used to measure monomeric anthocyanins by pH differential method (Lee et al. 2005) and for performing color analysis and HPLC/MS-MS analysis. After the first extraction, the pericarp was added to 20 mL of 2% formic acid and stirred at room temperature for 2 hr for a second extraction. The suspension from the second extraction was filtered and the filtrate was collected for further measurements. For comparison purposes, whole corn kernel samples were also analyzed for anthocyanin content. To extract anthocyanins from ground kernel, 2% formic acid was used.

5.2.4. Measurement of monomeric anthocyanin concentration

To measure monomeric anthocyanin concentrations, a pH differential method involving a microplate reader (Lee et al. 2005) and three independent replicates was used, as detailed in Section 4.2.4. (Chapter 4). The results were expressed as mg cyanidin-3-glucoside (C3G) equivalent/g pericarp.

5.2.5. Measurement of polyphenol concentration

Polyphenol concentrations were measured by using the Folin-Ciocalteu method adapted to a microassay (Heck et al. 2008). Samples were diluted by a factor of 1:40 with deionized water. In a 96 well plate, 50 µL of diluted samples with standard or blank (deionized water) were added. Another 50 µL of 1*N* Folin-Ciocalteu's phenol reagent was added to the mix. The process is detailed in Section 4.2.5. (Chapter 4). The results were expressed as mg gallic acid equivalent/g pericarp.

5.2.6. Measurement of flavonoid concentration

Flavonoid concentrations were measured according to the method reported by Bower et al. (2014). From (1:20) diluted samples, 50 µL samples were taken and rutin standards or blank (methanol) were added and placed in a 96 well plate. Then, 180 µL of methanol and 20 µL of 1% aminoethyl borinate in methanol were further added to the mix. Absorbance was read at 400 nm using a multi-well plate reader (Synergy 2, BioTek, Winooski, VT). The results were expressed as mg rutin equivalent/g pericarp.

5.2.7. Measurement of tannin concentration

Tannin concentrations were measured based on the method reported by Bindon et al. (2014) as described in Section 4.2.6. (Chapter 4). The results were expressed as mg catechin equivalent/g pericarp.

5.2.8. HPLC analysis

HPLC analysis of coproducts for the anthocyanin profiles was performed in triplicate using a Hitachi HPLC System (Hitachi High Technologies America, Inc., Schaumburg, IL) equipped with a multi wavelength detector and L-7100 pump. The method followed a previously reported protocol, with some modifications (West and Mauer 2013). The process is detailed in Section 4.2.7. (Chapter 4).

5.2.9. Mass spectrometry

MS-MS analysis was performed on a Waters quadrupole time-of-flight (Q-Tof) Ultima mass spectrometer equipped with an electrospray ionization (ESI) interface and controlled by MassLynx V4.1 software (Waters Corp., Milford, MA). The procedure is covered in detail in Section 4.2.8. (Chapter 4).

5.2.10. Tristimulus colorimetric measurement

The color was measured by using a Lovibond tintometer instrument (PFX990, The Tintometer Limited, Amesbury, England). The process is discussed in detail in Section 4.2.9. (Chapter 4).

5.2.11. Statistical analysis

All analyses were conducted with at least three independent replicates. SAS version 9.4 (SAS Institute, Cary, NY) was used for statistical analysis. Differences among independent variables were determined by using ANOVA, the proc GLM procedure and Tukey's test ($p < 0.05$). Pearson's correlation was calculated by using Office Excel (Microsoft Corporation, Redmond, WA).

5.3. Results and Discussion

5.3.1. Anthocyanin concentrations in steeping treatments and remaining pericarp

Anthocyanin concentrations from pericarp samples extracted with four treatments (water, water + SO_2, water + L.A., water + SO_2 + L.A.) and from untreated pericarp are shown in Fig. 5.1. The highest anthocyanin concentrations were observed in pericarp exposed to water + SO_2 + L. A. and water + SO_2 (22.9 and 20.5 mg C3G equivalent/g dry pericarp, respectively). Monomeric anthocyanin concentrations in steeping water containing L.A. were not different from the treatment containing only water, indicating that L.A. itself did not increase the extraction of anthocyanins from pericarp. Based on the amount of extracted anthocyanins obtained from dried pericarp after steeping (using 2% formic acid), we observed that anthocyanins remained in the pericarp. During the first extraction post steeping, the concentrations of anthocyanins in dried pericarp steeped with water, water + SO_2, water + L.A. and water + SO_2 + L.A. were 3.3, 8.0, 7.8 and 7.5 mg C3G equivalent/g dry pericarp, respectively (Fig. 5.1). The second extraction yielded lower concentrations of anthocyanins than the first extraction and the values were 0.6, 1.1, 1.4 and 1.4 mg C3G equivalent/g dry pericarp, respectively. Extracting anthocyanins directly from the untreated pericarp using 2% formic acid revealed a concentration of 22.5 mg C3G equivalent/g dry pericarp. The total anthocyanin concentration in the whole corn kernel after five sequential extractions was 4.9 mg C3G/g product (Fig. 5.2).

Total monomeric anthocyanin concentrations in steeping water from different processes, plus the remaining anthocyanins in the dried pericarp after treating with water, water + SO_2, water + L.A., water + SO_2 + L.A. and for untreated pericarp were 10.9, 29.5, 19.0, 31.9 and 28.0 mg C3G equivalent/g dry pericarp, respectively. The amount of monomeric anthocyanins in SO_2

+ L.A. treated steepwater was higher than amount of anthocyanins directly extracted with formic acid from the untreated pericarp. Therefore SO_2 combined with L.A. increased the extraction of anthocyanins from corn pericarp. Using sulfur salts resulted in 2000 ppm SO_2 and L.A., in the steepwater used in corn wet milling process, this combination yielded maximum total phenolics as well. The presence of SO_2 in steepwater increased the extraction of monomeric anthocyanins by 200% (Fig. 5.1).

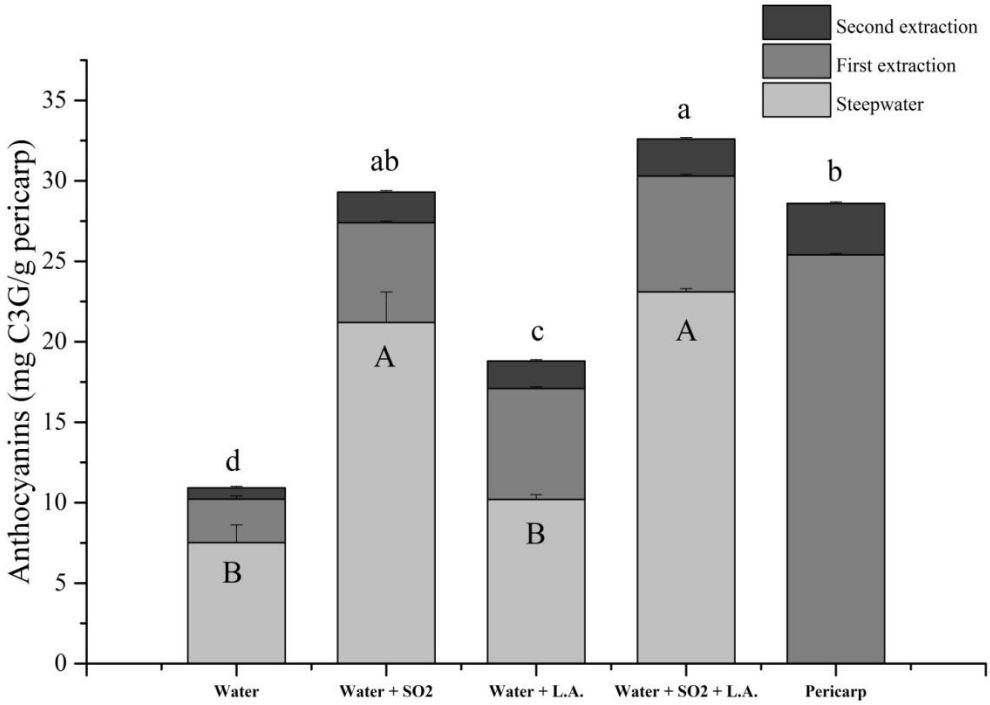


Fig. 5.1. Comparison of the amount of anthocyanins extracted from the pericarp steeping combinations and further extractions from the remaining dried pericarp with 2% formic acid. Mean ± SD from three replicates. Non-capital letters represent sum of anthocyanin concentrations from steeping water and subsequent pericarp extractions; capital letters represent anthocyanin concentrations from steeping water only. Bars with the same letters are not different ($p < 0.05$). Pericarp implies untreated pericarp.

A possible reason for the high extraction of anthocyanins by SO_2 is the interaction of anthocyanins with HSO_3^- ions leading to improved diffusion through cell walls and increased solubility of the pigments (Brouillard and Chahine 1980). HPLC chromatographs at 280 nm showed the presence of phenolic compounds. A “bump” in the HPLC chromatograph at 280 nm

is associated with the presence of proanthocyanidins (Nakamura et al. 2003); the bump was observed in this case for treatment with only water and water + L.A. groups (Fig. 5.3). However, no "bump" was present in the water + SO_2, or water + SO_2 + L.A. treatments, suggesting that SO_2 may play a role in the depolymerization of proanthocyanidins to smaller basic units, which resulted in increased anthocyanins.

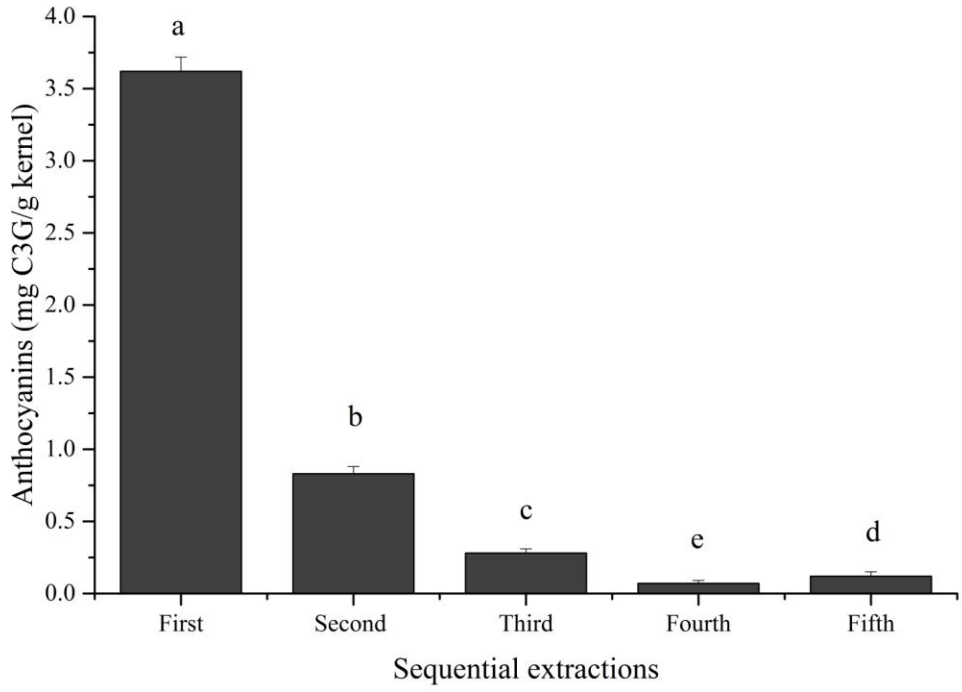


Fig. 5.2. Anthocyanin concentrations from whole corn kernel for five sequential extractions. Mean ± SD from three extractions. Bars with the same letter are not different ($p < 0.05$).

Due to the potential allergenicity, volatility and reactivity of SO_2, the FDA requires labeling of sulfites in certain foods when the concentrations are ≥ 10 ppm total SO_2

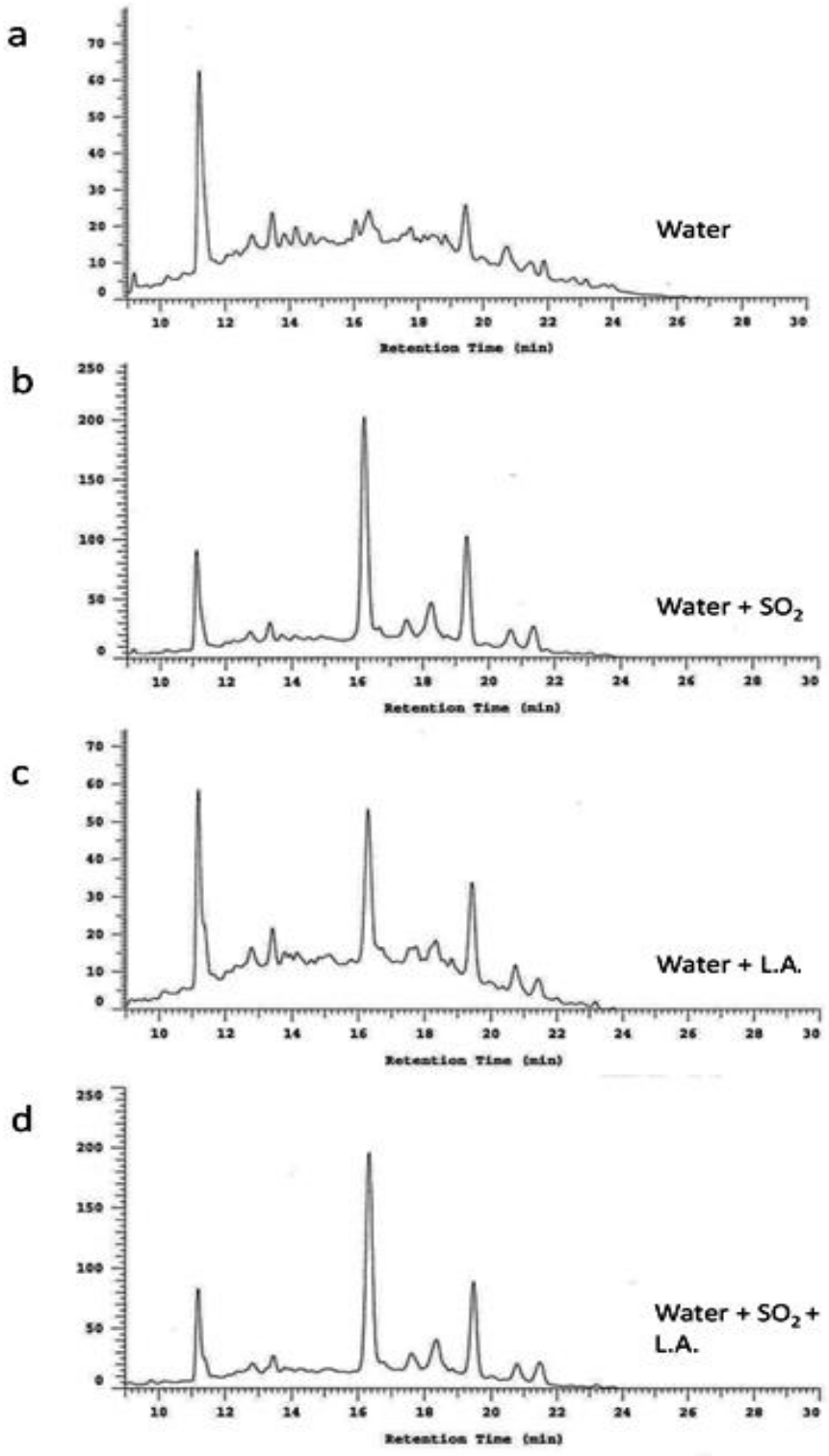


Fig. 5.3. HPLC chromatograms of corn pericarp steeping combinations with only water (a) water plus SO_2 (b) water plus L.A. (c) and water plus SO_2 and L.A. (d) at 280 nm.

(Timbo et al. 2004). Based on the final SO_2 concentrations in the finished product, it will be determined whether such product needs to be labeled.

5.3.2. Monomeric anthocyanin concentration in whole corn kernel

The first extraction of anthocyanins from whole corn kernel achieved 3.6 mg C3G/g kernel; the second extraction yielded 0.83 mg C3G/g anthocyanins (Fig. 5.2). The third and fourth extractions drew 7.8 and 1.9% of the first extraction, respectively. When 20% ethanol was used for the fifth extraction, it yielded 0.12 mg C3G/g kernel. Total anthocyanin concentration from five extractions was 4.9 ± 0.07 mg C3G equivalent/g kernel.

5.3.3. Polyphenol, flavonoid and tannin concentrations in steeping treatments

Polyphenol concentrations in the treatment with water + SO_2 were higher than the treatments with only water and water + L.A., the values being 47.1, 28.3 and 24.2 mg gallic acid equivalent/g dry pericarp, respectively (Fig. 5.4). Although polyphenols in the treatment with water + SO_2 were not different from the treatment with water + SO_2 + L.A., the mean of the polyphenol concentrations in the water + SO_2 treatment was higher than those of the treatments with water and water + L.A. For untreated pericarp, the polyphenol concentration in the formic acid extract was 53.0 mg gallic acid equivalent/g dry pericarp, which was higher than those extracted with water and water + L.A., but not different from treatments with water + SO_2 and water + SO_2 + L.A. Flavonoid concentrations did not differ among pericarp steeping treatments. However, the mean flavonoid concentration in pericarp extracted with formic acid was 23.0 ± 0.5 mg rutin equivalent/g dry pericarp, which was almost 100% greater than all other steeping treatments (Fig. 5.5).

Tannins have been reported to stabilize anthocyanins by synthesis of on anthocyanin derived pigments with higher stability (Alcalde-Eon et al. 2014). Tannin concentrations among

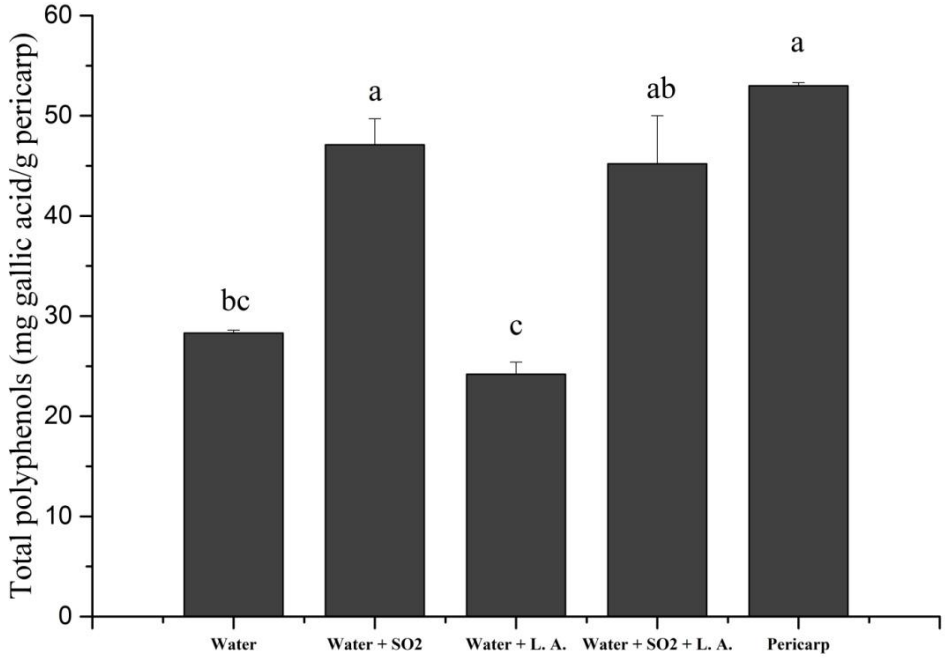


Fig. 5.4. Polyphenol concentrations from two extractions for treatments diluted with deionized water. Pericarp was extracted with 2% formic acid. Mean ± SD of three replicates. Bars with the same letter are not different ($p < 0.05$). Pericarp implies untreated pericarp.

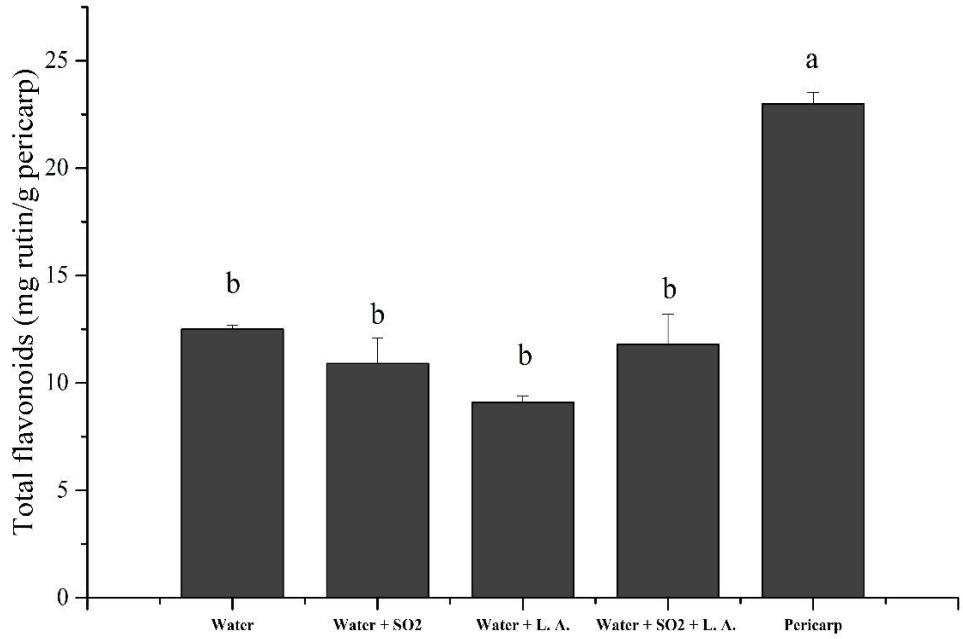


Fig. 5.5. Flavonoid concentrations from two extractions for treatments diluted with deionized water. Pericarp was extracted with 2% formic acid. Mean ± SD from three replicates. Bars with the same letter are not different ($p < 0.05$). Pericarp implies untreated pericarp.

different steeping treatments revealed a pattern similar to those of polyphenol concentrations (Fig. 5.6). Tannin concentrations were significantly higher in treatments containing SO_2 compared to treatments with water and water + L.A., indicating that SO_2 played a role in the potential depolymerization of the tannins as well. Again, formic acid extracts from untreated pericarp had the highest tannin concentration at 1093 µg catechin equivalent/g dry pericarp.

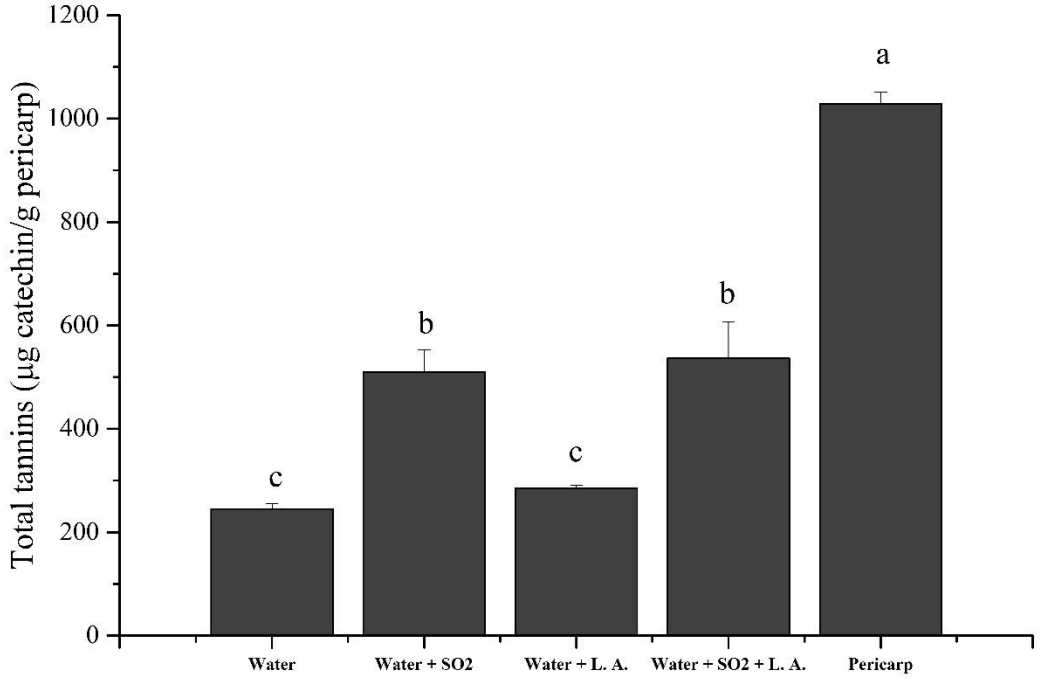


Fig. 5.6. Tannin concentrations from two extractions for treatments diluted with deionized water. Pericarp was extracted with 2% formic acid. Mean ± SD from three replicates. Bars with the same letter are not different ($p < 0.05$). Pericarp implies untreated pericarp.

5.3.4. Anthocyanin profiles of different steeping treatments

The types and amounts of individual anthocyanins in each steeping combination varied with the type of treatment. For treatment with only water, the predominant component was the condensed form at 37% (Fig. 5.7 a). However, in other the three treatments, water + SO_2, water + L.A. and water + SO_2 + L.A., the dominant anthocyanin present was C3G (Fig. 5.7 b, c and d).

The concentrations of different anthocyanins in steeping treatments are summarized in Table 5.1. All seven types of anthocyanins, including the condensed form, non-acylated and acylated cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucosides were higher in

TABLE 5.1. Anthocyanin concentrations in steeping treatments detected by HPLC at 520 nm.

	Concentration of anthocyanins (µg/mL)			
Anthocyanin	**Water**	**Water + SO_2**	**Water + L.A.**	**Water + SO_2 + L.A.**
Condensed form	13.5 ± 0.1d	22.1 ± 0.1a	14.4 ± 0.2c	20.7 ± 0.02b
Cyanidin-3-Glc	3.0 ± 0.1c	107.5 ± 0.4a	25.6 ± 0.9b	104.4 ± 1.0a
Pelargon-3-Glc	0.5 ± 0.1d	13.4 ± 0.4a	2.7 ± 0.09c	10.8 ± 0.8b
Peonidin-3-Glc	1.8 ± 0.1d	35.4 ± 0.9a	9.3 ± 0.4c	30.9 ± 1.0b
Cy3-6”Malonylglc	7.4 ± 0.1d	55.6 ± 1.6a	15.7 ± 0.6c	48.9 ± 0.8b
Pg3-6”Malonylglc	0.7 ± 0.1d	12.2 ± 0.5a	3.2 ± 0.2c	10.3 ± 0.2b
Pn3-6”Malonylglc	3.1 ± 0.1d	18.7 ± 0.2a	5.3 ± 0.2c	16.9 ± 0.1b

Mean ± SD from three replicates; Means followed by the same letter in one row are not different ($p < 0.05$).

the treatment with water + SO_2 and water + SO_2 + L.A. compared to the treatments with only water and water + L.A. The concentration of C3G in treatments with water + SO_2 (107.5 µg/mL) and water + SO2 + L.A. (104.4 µg/mL) were more than 30 times higher than the concentration in the steeping treatment with only water (3.0 µg/mL). The C3G concentration in the treatment with water + L.A. (25.6 µg/mL) was almost ten times higher than in the treatment with only water; although the concentrations were not as high as those in treatments involving SO_2. Proanthocyanidins concentrations were higher in only the water and water + L.A. combinations, but they were lower in treatments with SO_2 (Fig. 5.3).

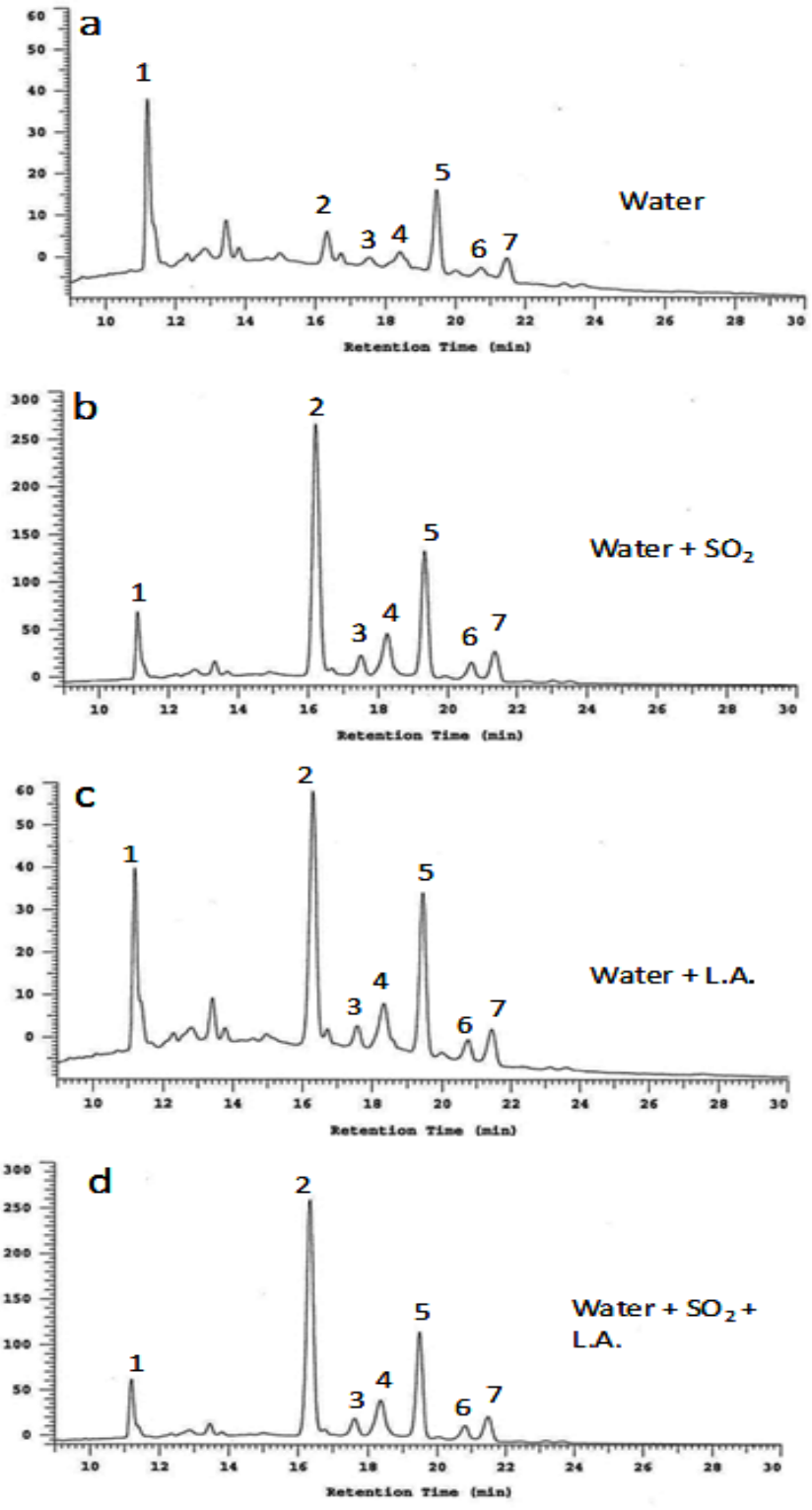


Fig. 5.7. HPLC chromatographs for all treatments at 520 nm. The peaks indicate the following: 1: Condensed form; 2: C3G; 3: P3G; 4: Peo3G; 5: C36MG; 6: P36OMG and 7: Peo36OMG.

5.3.5. Effect of SO_2 and lactic acid treatments on extract color

Color parameters of all pericarp steeping combinations were measured at different dilution factors. SO_2 and L.A. affected the original color of the steeping water (Table 5.2). Further dilutions changed the hue angle, chroma and color difference of the extracts. The reason that L.A. increased chroma is related to the lowering of the solution pH. An increase in pH value results in reduced color intensity of red wines (Sims and Morris 1984). The pH values of the water and water + SO_2 treatments were not different (4.58); however, the presence of L.A., regardless of the presence of SO_2, lowered the pH (3.37). The color change after dilution was due to the change in concentration of isolated pigments rather than pH. The smallest color difference (ΔE) between individual samples and the pericarp directly extracted with 2% formic acid was 4.85 in the treatment with water + L.A. at 1:4 dilution. However, further dilutions in the presence of SO_2 enhanced the color differences, which could be attributed to the bleaching effect produced by SO_2 (Boulton 2001). Researchers have reported that although addition of SO_2 results in greater anthocyanin extraction, however, this is often at the expense of reversible bleaching due to formation a stable and colorless compound named anthocyanin-4-bisulfite (Picinelli et al. 1994).

Chroma was correlated with the concentration of anthocyanins (r = 0.99) and tannins (r = 0.99). Hue angle and Saturation were not correlated with any of the variables that were measured in the pericarp steeping combinations (Table 5.2). The anthocyanin distributions (%) in the pericarp steeping combinations for only water (a), water + SO_2 (b), water + L.A. (c) and water + SO_2 + L.A. (d) are summarized in Fig. 5.8.

TABLE 5.2. Color parameters of pericarp steeping treatments and correlations among color parameters and monomeric anthocyanins, polyphenols, tannins and flavonoids.

Sample	Dilution Factor	L*	a*	b*	Hue angle	Chroma	ΔE*	Color square
Water	1:1	2.02 ± 0.01d	0.23 ± 0.01d	-1.10 ± 0.01d	281.80 ± 0.55a	1.12 ± 0.01d	59.9 ± 0.01a	
	1:2	4.46 ± 0.01c	10.37 ± 0.05c	1.57 ± 0.01c	8.58 ± 0.02 d	10.48 ± 0.05c	49.6 ± 0.05b	
	1:4	15.96 ± 0.03b	34.26 ± 0.05b	18.22 ± 0.04b	27.99 ± 0.02 c	38.80 ± 0.06b	18.5 ± 0.07d	
	1:8	37.65 ± 0.03a	37.29 ± 0.04a	22.20 ± 0.03a	30.76 ± 0.21b	43.40 ± 0.05a	21.9 ± 0.01c	
Water +SO_2	1:1	17.36 ± 0.08d	30.30 ± 0.11a	20.26 ± 0.12b	33.80 ± 0.07c	36.45 ± 0.16b	20.8 ± 0.16d	
	1:2	37.45 ± 0.03c	30.45 ± 0.01a	26.18 ± 0.01a	40.70 ± 0.03a	40.15 ± 0.01a	25.6 ± 0.03c	
	1:4	56.90 ± 0.01b	22.53 ± 0.03b	19.22 ± 0.01c	40.47 ± 0.04a	29.61 ± 0.03c	46.3 ± 0.02b	
	1:8	71.35 ± 0.05a	15.62 ± 0.02c	12.25 ± 0.01d	38.09 ± 0.05b	19.85 ± 0.01d	63.2 ± 0.05a	
Water+ L.A.	1:1	3.97 ± 0.01d	8.17 ± 0.07d	0.86 ± 0.02a	5.98 ± 0.12c	8.21 ± 0.08d	51.0 ± 0.08a	
	1:2	11.39 ± 0.01c	31.77 ± 0.02c	12.12 ± 0.01b	20.90 ± 0.01b	34.00 ± 0.02c	25.2 ± 0.01b	
	1:4	19.89 ± 0.04b	44.65 ± 0.06b	26.88 ± 0.09c	31.05 ± 0.05a	52.11 ± 0.10b	4.9 ± 0.08d	
	1:8	38.97 ± 0.01a	57.03 ± 0.01a	33.97 ± 0.01d	30.78 ± 0.01a	66.37 ± 0.02a	21.2 ± 0.01c	
Water +SO_2+L.A.	1:1	18.91 ± 0.23d	37.82 ± 0.34c	24.00 ± 0.36b	32.40 ± 0.20b	44.79 ± 0.48b	12.3 ± 0.46d	
	1:2	35.77±0.03c	44.21 ± 0.04a	28.58 ± 0.05a	33.02 ± 0.01a	52.64 ± 0.06a	16.4 ± 0.04c	
	1:4	54.05±0.04b	39.49 ± 0.08b	20.00 ± 0.01c	27.02 ± 0.03c	44.27 ± 0.08b	36.1 ± 0.06b	
	1:8	68.41±0.02a	29.86 ± 0.03c	11.88 ± 0.02c	21.80 ± 0.01d	32.13 ± 0.04c	54.4 ± 0.04a	

L*: lightness of the color; **a***: position of the color between red/magenta and green; **b***: position of the color between yellow and blue; Hue angle = arctan (b*/a*); Chroma = sqrt ($a^{*2} + b^{*2}$); $\Delta E^* = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$; L_0, a_0, b_0 represent the color of pericarp directly extracted with 2% formic acid. Means followed by the same letter in one column are not different.

	Correlation coefficients			
	Anthocyanins	**Polyphenols**	**Tannins**	**Flavonoids**
Hue angle	0.611 (*p*= 0.389)	0.344 (*p*= 0.656)	0.550 (*p*= 0.450)	0.844 (*p*= 0.156)
Chroma	0.999 (*p*= 0.0006)	0.933 (*p*= 0.067)	0.994 (*p*= 0.00057)	0.079 (*p*= 0.921)
Saturation	0.760 (*p*= 0.240)	0.508 (*p*= 0.492)	0.704 (*p*= 0.296)	0.706 (*p*= 0.294)

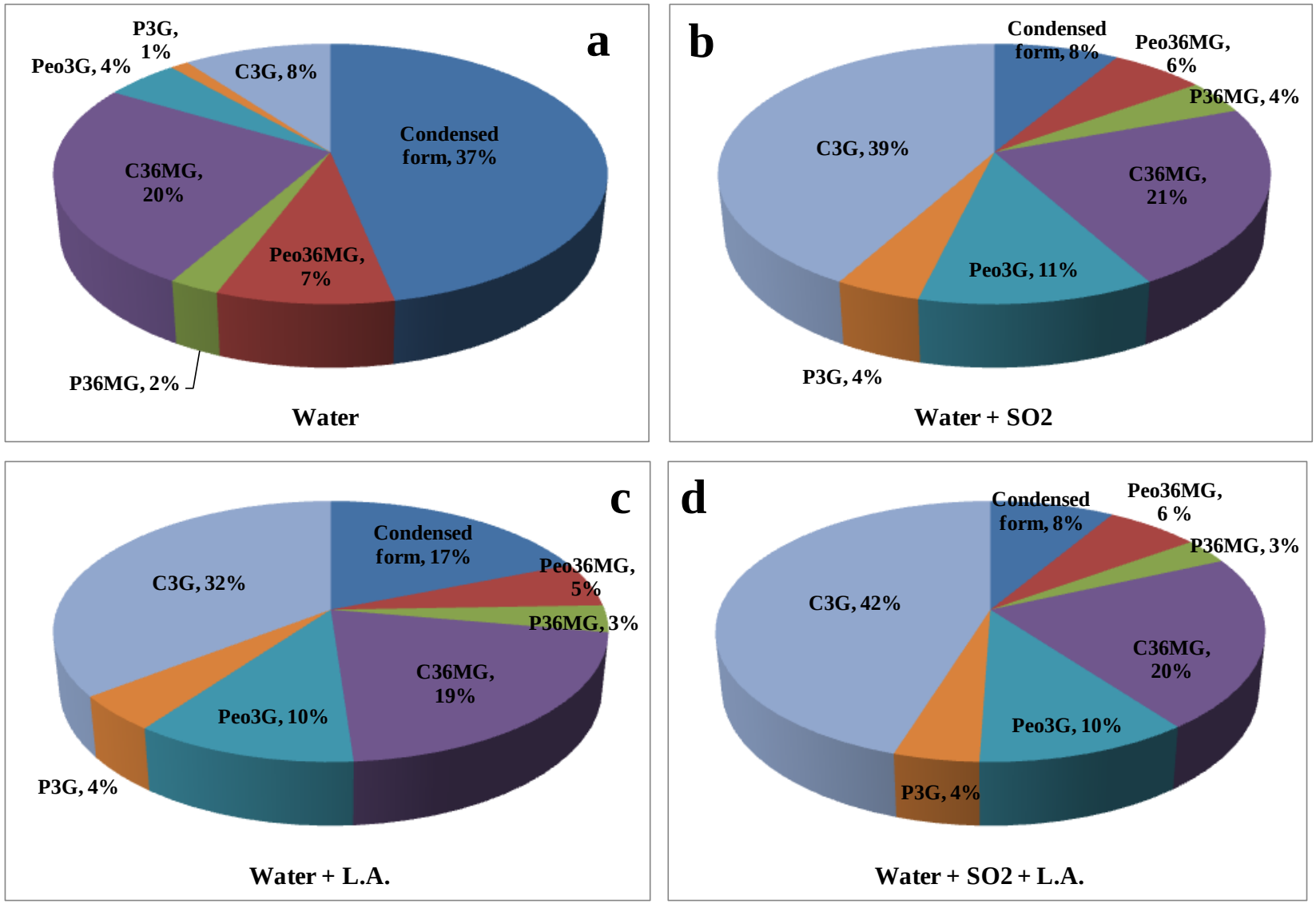


Fig. 5.8. Anthocyanin distribution (%) in steeping water from the treatments as determined by HPLC at 520 nm.

5.4. Conclusions

Steeping water with SO_2 contained monomeric anthocyanin equivalents of 20.5 mg C3G/g dry pericarp, which was higher than the treatment containing only water (7.1 mg C3G/g dry pericarp). The treatment with a combination of SO_2 and L.A. had the greatest monomeric anthocyanin concentration (22.9 mg C3G/g dry pericarp). This was associated with increased C3G and condensed forms of anthocyanins. Chroma was correlated with the concentration of anthocyanins (r = 0.999) and tannins (r = 0.994). It was found that the presence of SO_2 in the steeping water improved anthocyanin extraction from purple corn pericarp; however, it had a bleaching effect on the final color. In the treatment containing only water for steeping, the condensed forms were the most abundant form of anthocyanins. However, the predominant anthocyanin form in treatments with SO_2/L.A. and the combination of SO_2 and L.A. was cyanidin-3-glucoside. Total 23 g of anthocyanins/kg purple corn pericarp were quantified, making it one of the richest sources of anthocyanins on a w/w basis. A novel procedure to enhance total anthocyanin extraction from purple corn pericarp has been proposed.

Chapter 6. A 100 g corn dry milling protocol for lab scale measurement of coproduct yield and composition

6.1. Background information and motivation

Corn dry milling involves fractionating the kernel into different sizes containing endosperm, germ and pericarp. Based on particle size, endosperm fractions are further classified into flaking grits, cones, meal and flour. Large grits are used to make breakfast cereals and command a high market price compared to other dry milling coproducts (Yuan and Flores 1996). Unlike wet milling and dry grind processes where soft endosperm corn is preferred, hard endosperm corn is the choice for dry milling process as it yields greater proportion of large grits (Paulsen and Hill 1985). Remaining endosperm fractions are utilized as brewery adjuncts and for making corn flour. Germ and pericarp are combined with some endosperm fractions to make hominy feed, a low value coproduct (Rausch and Belyea 2006). Corn dry milling mainly caters to the needs of the human food sector; it is a relatively small industry and processes less than 2% of the total US corn (CRA 2016). Generally dry milling characteristics of corn (fraction yields and their compositions) vary widely among corn cultivars (Rausch et al. 2009).

In the absence of necessary enzymes, humans cannot digest pericarp fiber; to prevent lipid oxidation and increase shelf life, <1% fat is desired in endosperm fractions. Tempering corn is a vital operation whose purpose is to hydrate the germ, making it resilient against breakage during subsequent processing. Therefore, tempering conditions (time, temperature and moisture) and degermination method are important considerations and determine the effectiveness of the whole process (Rausch et al. 2009). Tempering is a vital dry milling operation; it helps in achieving differential hydration of corn kernels, making the germ resilient and facilitates

effective germ and endosperm separation. Various methods used by researchers include two to three step tempering operations and tempering times of up to 16 hr (Katta et al. 1987; Kirleis and Stroshine 1990; Pan et al. 1996; Stroshine 1986). In a lab scale study (12 kg samples), Peplinski et al. (1984) reported that a single-stage tempering method yielded large grits with 1.0 to 1.6% fat content. Effectiveness of a single stage tempering method was demonstrated in a series of pilot plant scale studies (40 kg samples) wherein a single stage 10 min tempering process was found to be sufficient for high flaking grit yields, although the fat content of flaking grits was not ascertained (Mehra and Eckhoff 1997; Mehra et al. 2001). Rausch et al. (2009) reported a scaled down (1 kg), single stage tempering procedure which gave milling yields and crude fat contents comparable to those in the industry. However, due to a roller milling step required for germ separation, it was not possible to recover "true sized" large grits, although the process precisely quantified large grit yields.

During this project we explored the viability of using colored corns for economic anthocyanin recovery. The plant breeding group at the University of Illinois concentrated on developing new colored corn cultivars with higher pigment contents and adapting them to climatic conditions of the Midwestern US. Several types of colored corn were bred; often their harvested quantities ranged from a few hundred grams to a few kilograms. Therefore, there was a need to dry mill corn samples at 100 g scale and recover coproducts for anthocyanin content determination. A study was conducted with 100 g samples and processing them using the scaled down version of 1 kg scale protocol (Rausch et al. 2009). Coproduct yields were comparable with the 1 kg process when post-temper drying time was reduced from 2 hr to 30 min in 49°C oven (Table 6.2). This demonstrated feasibility of a 100 g dry milling protocol; however, further adjustment of tempering time and post-temper drying duration was required. Another challenge

was to adapt the process to recover large grits of size comparable to the commercial large grits so that not only yields, but also coproduct size from the protocol corresponded to those of industry.

6.2. Materials and Methods

6.2.1. Materials

Ten corn cultivars, including six yellow, three colored and one white, were processed. Yellow corn included three hard endosperm cultivars (Hard1, Hard2 and Hard3), one high extractable starch (HE), one high amylose (HA) and one waxy cultivar (Waxy). All yellow corn and white corn were sourced from a major seed supplier. Colored corn cultivars included purple (Purple), blue (Blue) and red (Red). Purple corn was procured from a specialty foods vendor (Lot No. L670106, Angelina's Gourmet, Swanson, CT). Jerry Peterson Blue Organic corn (Lot No. 41633) was purchased from Johnny's Selected Seeds (Fairfield, ME). Red corn was purchased from an online vendor (Item model number: G64C63, Amazon.com, Inc., Seattle, WA). Corn was cleaned using a 12/64" (4.8 mm) sieve for removal of broken corn and foreign material (BCFM). Coproduct moisture contents were measured using an air oven at 135°C for 2 hr (Approved Method 44-15A, AACC International 2010).

Kernel physical properties were determined using standard procedures. Test weight (TW) was measured using standard apparatus (Approved Method 55-10, AACC International 2010) and was expressed in kilograms per hectoliter (kg/hL). Absolute density (AD) was determined using the ethanol column test (Hill et al. 1990). Thousand-kernel weight (TKW) was measured according to a method reported by Groos et al. (2003). All physical properties were measured in triplicate.

Compositional analyses of the corn and coproducts were done at a commercial analytical laboratory (Illinois Crop Improvement Association, Champaign, IL). Analyses included crude protein (Method 990.03, AOAC 2003), crude fat (Method 920.39, AOAC 2003) and neutral detergent fiber content (Van Soest et al. 1991). Starch contents of corn were measured using an acid hydrolysis method described by Vidal et al. (2009). All analyses were done in duplicate.

6.2.2. Moisture content determination and tempering

Moisture content of corn sample was ascertained using an electronic moisture tester (GAC II, Dickey-John, Auburn, IL). The amount of water required to increase the kernel moisture content to 23.5% (wet basis) was calculated. Corn samples (100 g) and the required amount of tap water at room temperature were added to 4 L cylindrical plastic bottles and sealed with caps. Corn samples were tempered by rotating the bottles continuously at 0.5 rpm for 30 min at room temperature until water was fully absorbed by corn kernels.

6.2.3. Preliminary 100 g study

Initial 100 g experiments were conducted with a hard endosperm cultivar (Hard1) using 1 kg protocol (Rausch et al. 2009). Corn samples (100 g) were mixed with water and tempered in plastic bottles for 20 min. Post tempering, the material was passed through a horizontal drum degerminator and conditioned in 49°C oven for 2 hr. After conditioning; sieving, roller milling and aspiration steps were used to separate large grits, small grits, fines, pericarp and germ fractions. Further experiments were conducted by reducing the post-temper drying times to 1 hr and 30 min.

6.2.4. Modification study for 100 g dry milling protocol

Based on the insights gained from preliminary 100 g experiments using the 1 kg protocol, it was realized that tempering duration and post-temper drying time were the most important parameters which need to be adjusted. As a result, a 3x3 factorial design experiment was planned with three tempering times (20, 30 and 40 min) and three post-temper drying times (30 min, 1 hr and 2 hr). While tempering hard endosperm cultivars, it was observed that 20 min tempering time was not sufficient and unabsorbed water remained in the tempering vessel. There was also a need to identify ideal post-temper drying time for 100 g samples. All experiments were done in triplicate using Hard1 cultivar of hard endosperm corn.

6.2.5. The 100 g dry milling protocol

After tempering tempering to increase the kernel moisture content to 23.5%, corn was fractionated using a lab scale horizontal drum degerminator (15.2 cm diameter concentric roll, 1275 rpm and 375 W motor). Degermination process involves breaking of corn kernels by applying a shear force, so as to free endosperm fractions from the germ, which largely remain intact due to relatively higher levels of hydration. Fractionated corn was conditioned in a convection oven at 49°C for 1 hr. Conditioned samples were sieved over a 5 mesh sieve (4.0 mm openings and 20.32 cm diameter) using a sieve shaker for 1 min (Model RX-86, W. S. Tyler, Inc., Mentor, OH). Subsequent sieving operations were carried out using the same sieve shaker. Two streams were created, one that stayed on the sieve (+5) and the one that passed through the sieve (-5). The use of (+X) in subsequent discussion implies the fraction that remained on the sieve while (-X) implies the fraction which passed through (Fig. 6.1).

The +5 stream consisted of large grits, germ and pericarp fractions. An aluminum pan was used and germ fractions were recovered manually after visual identification. Any pericarp fractions sticking to the germ also were separated manually. This separation process required 25

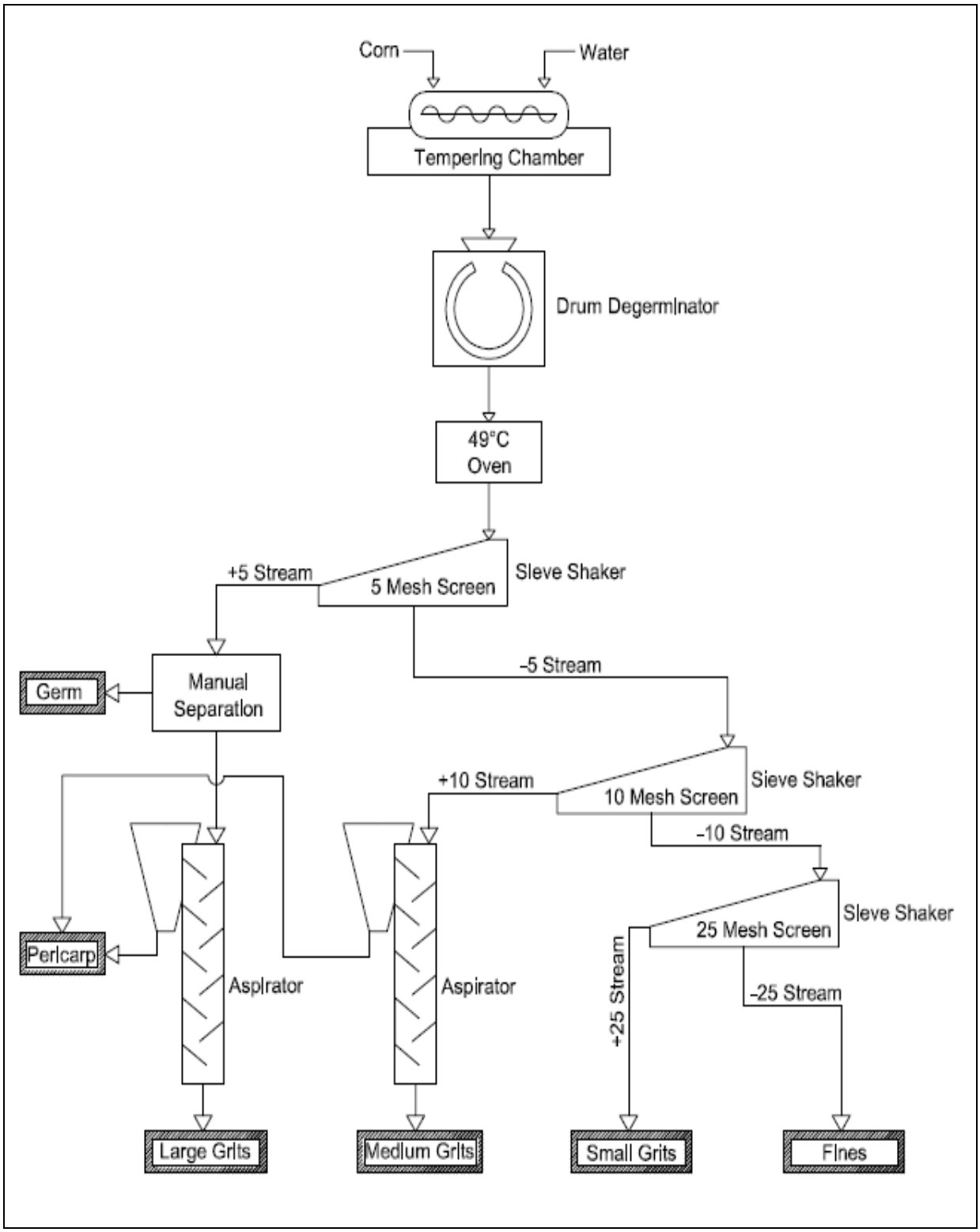


Fig. 6.1. Process flow in the 100 g dry milling protocol.

min. Remaining endosperm and pericarp fractions were placed on a 6/64" (2.38 mm) round hole sieve with a catch pan placed underneath. Contents were rubbed with a Scotch-Brite® scrub sponge (The 3M Company, Maplewood, MS) for 1 min and fractions which passed through were collected as pericarp. The material which remained on top of the sieve was aspirated twice using a lab scale aspirator (Model 6DT4, Kice Metal Products, Wichita, KS) operating at 5 mm H_2O negative pressure to separate pericarp from large grits.

The -5 stream which consisted of remaining endosperm fractions and pericarp, was sifted on a 10 mesh sieve (1.68 mm openings) for 1 min. The +10 stream was aspirated twice to separate medium grits and pericarp which was added to the pericarp fractions already recovered from the +5 stream. The -10 stream was sifted further on a 25 mesh sieve (0.71 mm openings) for 1 min. The +25 stream was collected as small grits and the -25 stream constituted fines. The modification study, discussed in Section (6.2.4) was used to ascertain various parameters used in this protocol.

6.2.6. Statistical analysis

Each corn type was processed three times. Analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests were conducted using SAS Studio (SAS Institute, Cary, NY). The level selected to detect difference among means was 5% ($p < 0.05$).

6.3. Results and Discussion

6.3.1. Physical and compositional characteristics of corn cultivars

Physical and compositional characteristics of ten different corn cultivars are summarized in Table (6.1). Highest TKW was observed for red corn (526 g) while the lowest value was identified in high amylose corn (273 g). Hard endosperm corn cultivars had higher TKW

TABLE 6.1. Physical and compositional corn characteristics.

	Physical characteristics				Compositional characteristics (% db)			
Cultivar	**Moisture Content (%)**	**Thousand-kernel weight (g)**	**Test weight (kg/hL)**	**Absolute density (g/mL)**	**Starch**	**Crude protein content**	**Crude oil content**	**Neutral detergent fiber**
Hard1	12.3	382.22 ± 2.42c	95.93 ± 0.39a	1.28 ± 0.01b	73.72 ± 0.38de	8.78 ± 0.15de	4.03 ± 0.13e	8.80 ± 0.15d
Hard2	12.3	401.67 ± 6.65b	94.46 ± 0.84b	1.30 ± 0.02ab	73.95 ± 0.12d	9.84 ± 0.42bc	4.34 ± 0.03e	9.23 ± 0.31cd
Hard3	12.2	342.35 ± 7.16ef	95.40 ± 0.14ab	1.32 ± 0.03a	76.98 ± 0.41a	8.79 ± 0.05de	3.91 ± 0.05e	7.81 ± 0.25e
HE	12.5	355.17 ± 7.35de	89.07 ± 0.18e	1.23 ± 0.01c	75.21 ± 0.14c	8.44 ± 0.04ef	3.28 ± 0.01f	8.03 ± 0.17e
HA	13.3	273.85 ± 3.01h	78.88 ± 0.85g	1.15 ± 0.01d	67.70 ± 0.05f	9.94 ± 0.35b	5.88 ± 0.05a	24.65 ± 0.46a
Waxy	12.9	328.52 ± 2.99fg	90.95 ± 0.56d	1.23 ± 0.01c	76.09 ± 0.31b	7.96 ± 0.32f	3.09 ± 0.04f	10.01 ± 0.15bc
Purple	14.5	359.19 ± 3.13d	72.55 ± 0.30i	1.06 ± 0.02e	63.44 ± 0.13h	10.31 ± 0.02b	4.74 ± 0.03c	9.44 ± 0.18cd
Blue	16.2	313.51 ± 12.75g	87.46 ± 0.17f	1.18 ± 0.01d	66.53 ± 0.11g	11.16 ± 0.08a	5.69 ± 0.04a	9.70 ± 0.04bc
Red	14.8	526.60 ± 13.00a	74.84 ± 0.42h	1.06 ± 0.01e	74.24 ± 0.14d	9.23 ± 0.05cd	5.32 ± 0.05b	9.81 ± 0.35bc
White	15.5	336.32 ± 4.66f	92.64 ± 0.51c	1.29 ± 0.01ab	73.06 ± 0.22e	9.79 ± 0.05bc	4.09 ± 0.04de	10.29 ± 0.23b

Physical characteristics: Mean ± SD from three replicates; Compositional characteristics: Mean ± SD from two replicates; Means followed by the same letter in a column are not different ($p < 0.05$).
Hard1, Hard2 and Hard3: hard endosperm cultivars; HE: high extractable starch corn; HA: high amylose corn; Waxy: waxy corn; Purple, Blue and Red: colored corn cultivars; White: white corn.

compared to the colored cultivars. Hard1 cultivar had highest TW (95.9 kg/hL) while purple corn had the lowest value (72.5 kg/hL). Hard endosperm cultivars of corn had highest TW. AD varied from 1.32 to 1.06 g/mL with Hard3 and purple/red corn cultivars having highest and lowest values, respectively. As in case of TKW and TW values, hard endosperm cultivars of corn had highest AD values.

Starch content of corn varied from 76.9 to 63.4% (db), respectively, with hard endosperm cultivar Hard3 having the highest and Purple with the lowest amount. Crude protein content was highest for Blue and lowest for Waxy with values being 11.1 and 7.9% (db), respectively. HA corn had the highest crude oil content 5.8% (db) while the lowest value was detected in Waxy 3.09% (db). Neutral detergent fiber values varied from 24.7 to 7.8% (db), respectively, with HA corn having the maximum and the Hard3 cultivar of hard endosperm corn having the minimum values.

6.3.2. Preliminary 100 g study using 1 kg protocol

With a post-temper drying time of 2 hr, the average germ yield in 100 g experiments was only 2.84% (db) as compared to 10.01% (db) for 1 kg scale experiments with same corn (Table 6.2). Conversely, the large grits and fines yield were higher for the 100 g runs. When post-temper drying time was reduced to 1 hr, it resulted in a marginal increase of germ recovery (3.75% db). However, when the post-temper drying time for 100 g runs was reduced further to 30 min, germ recovery increased to 9.40% (db) and all fraction yields from both 100 g and 1 kg runs were similar. In absence of a lab scale gravity separator, the 1 kg protocol used a roller milling step to flatten the hydrated germ which resisted breakage unlike the endosperm fractions; flattened germ was separated from endosperm using a 10 mesh screen. However in 100 g experiments, 2 hr post-temper drying time resulted in higher moisture loss from germ, leaving it

susceptible to breakage during subsequent roller milling step which was reflected in low yields of germ and higher yields of endosperm fractions.

TABLE 6.2. Coproduct yields from preliminary study with Hard1 corn (% db).

Scale (Temp x dry)	**1 kg** (20 min x 2 hr)	**100 g** (20 min x 2 hr)	**100g** (20 min x 1 hr)	**100 g** (20 min x 30 min)
Large grits	35.49 ± 0.26b	39.16 ± 1.78a	39.15 ± 0.86a	35.76 ± 1.78b
Small grits	27.82 ± 0.25a	26.61 ± 1.38a	26.68 ± 0.89a	26.61 ± 1.38a
Fines	21.27 ± 0.22b	27.66 ± 0.63a	27.23 ± 1.86a	22.59 ± 0.63b
Germ	10.01 ± 0.09a	2.84 ± 0.38c	3.75 ± 0.52b	9.40 ± 0.38a
Pericarp	3.84 ± 0.03b	3.27 ± 0.13c	3.21 ± 0.20c	4.43 ± 0.13a
Total	99.44 ± 0.48a	99.56 ± 0.32a	100.03 ± 0.43a	99.92 ± 0.32a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$). Tempering x post-temper drying times.

6.3.3. Modification of 100 g dry milling procedure

A tempering time of 20 min was sufficient for colored corn cultivars to absorb water but for hard endosperm cultivars, unabsorbed water remained in the tempering bottle. However, 30 min tempering time was sufficient and there was no difference if tempering was extended further to 40 min. Since the modified protocol did not involve a roller milling step to ensure recovery of true sized large grits, post-temper drying times of 2 hr, 1 hr and 30 min were selected to determine the best combination.

Highest yields of flaking grits (42.6%) were obtained with a combination of 20 min tempering and 30 min post-temper drying time. However, 20 min tempering was not sufficient and water remained unabsorbed in the tempering bottle. Since earlier studies (Rausch et al. 2009;

Mehra et al. 2001) stressed the importance of having desired processing moisture content, it was decided to proceed with a longer tempering time of 30 min in the protocol. Next higher yields of large grits were recorded in treatments with 30 min post-temper drying times. However, in treatment involving 30 min tempering and 30 min post-temper drying time, total yield was only 94.6% (db). This behavior was observed in other tests involving this combination (data not presented). This was due to the fact that 30 min post-temper drying time was not sufficient to dehydrate the samples and moisture was lost during subsequent processing. Although minuscule, this moisture loss adversely affected the final mass balance due to the smaller scale of the process. In treatments with 1 and 2 hr post-temper drying times, coproduct yields were not different. As a result, 1 hr post-temper drying time was found optimal for 100 g samples. To facilitate effective hydration of the corn kernel, which in turn ensured effective endosperm, germ and pericarp separation, tempering time of 30 min was selected.

6.3.4. Repeatability of the 100 g procedure

To test the protocol consistency, 12 milling runs were conducted with a hard endosperm cultivar of corn (Hard1) using 30 min tempering and 1 hr post-temper drying time. Six coproducts (large grits, medium grits, small grits, fines, germ and pericarp) recovered from the process are depicted in Fig. 6.2. Total recovery varied between 100.91 and 99.15% (db) with a low coefficient of variation (COV) of 0.50% (Table 6.4). Large grits yield varied from 42.82 to 39.77% (db) with a standard deviation of 1.06. Yield of medium grits (+10 stream) varied between 31.06 and 27.45% (db) with a standard deviation of 1.06. Small grits yield (+25 stream) varied between 8.78 and 8.07% (db) with a standard deviation of 0.22. Fines fraction (-25 stream) yields ranged from 5.11 to 3.92% (db) with a standard deviation of 0.42. Mean yields for

TABLE 6.3. Coproduct yields for tempering and post-temper drying combinations at 100 g scale with Hard1 corn (% db).

	Tempering (20 min)			Tempering (30 min)			Tempering (40 min)		
Drying[1]	**30 min**	**1 hr**	**2 hr**	**30 min**	**1 hr**	**2 hr**	**30 min**	**1 hr**	**2 hr**
Large grits	42.60 ± 0.13a	37.25 ± 1.77c	38.30 ± 2.10bc	37.88 ± 1.16bc	38.38 ± 0.79bc	38.22 ± 1.91bc	41.75 ± 1.64a	37.97 ± 0.38bc	40.47 ± 0.83ab
Medium grits	27.90 ± 0.38b	32.11 ± 1.50a	31.82 ± 1.04a	28.35 ± 1.12b	31.56 ± 1.63a	31.92 ± 2.32a	27.92 ± 0.99b	31.03 ± 1.58a	29.91 ± 1.11ab
Small grits	8.47 ± 0.11ab	8.84 ± 0.14ab	9.00 ± 0.12a	8.61 ± 0.06ab	8.82 ± 0.19ab	8.31 ± 0.10ab	8.11 ± 1.03b	8.76 ± 0.30ab	8.24 ± 0.04ab
Fines	4.30 ± 0.33a	4.89 ± 0.37a	4.84 ± 0.34a	4.54 ± 0.40a	4.27 ± 0.26a	4.48 ± 0.42a	4.61 ± 0.36a	4.67 ± 0.41a	6.63 ± 0.24a
Germ	12.09 ± 0.06ab	12.10 ± 0.51ab	12.05 ± 0.26ab	10.26 ± 2.62b	12.44 ± 0.26a	11.96 ± 0.49ab	12.65 ± 0.68a	12.42 ± 0.25a	12.86 ± 0.07a
Pericarp	4.73 ± 0.17a	4.72 ± 0.69a	4.69 ± 0.26a	4.93 ± 0.12a	5.00 ± 0.05a	5.01 ± 0.09a	5.10 ± 0.06a	5.02 ± 0.12a	5.05 ± 0.03 a
Total	100.12 ± 0.55a	99.94 ± 0.44a	100.73 ± 0.48a	94.60 ± 1.00b	100.50 ± 0.42a	99.91 ± 0.12a	100.16 ± 1.26a	99.88 ± 0.65a	101.18 ± 0.18a

Mean ± SD from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$). [1]Post-temper drying. Hard1, Hard2 and Hard3: hard endosperm cultivars; HE: high extractable starch corn; HA: high amylose corn; Waxy: waxy corn; Purple, Blue and Red: colored corn cultivars; White: white corn.

TABLE 6.4. Coproduct yields from repeatability studies with Hard1 corn (% db).

Replicate	1	2	3	4	5	6	7	8	9	10	11	12	Mean ± SD	[1]COV %
Large grits	42.82	40.10	42.19	39.93	41.04	41.99	39.77	42.18	41.18	39.85	40.32	40.05	40.95 ± 1.06	2.57
Medium grits	27.45	29.86	28.79	31.06	28.73	28.54	29.54	27.74	29.82	29.67	29.67	30.87	29.31 ± 1.06	3.62
Small grits	8.37	8.14	8.07	8.53	8.56	8.19	8.56	8.05	8.28	8.38	8.33	8.78	8.35 ± 0.22	2.57
Fines	5.11	4.13	4.00	4.83	4.31	4.09	4.88	3.92	3.99	5.01	4.16	4.16	4.38 ± 0.42	9.64
Germ	11.51	12.60	12.66	11.70	11.84	13.21	12.15	12.46	12.40	12.52	12.32	12.19	12.30 ± 0.44	3.60
Pericarp	5.20	5.59	4.74	4.65	4.66	4.89	5.31	5.29	5.21	4.76	5.00	4.59	4.99 ± 0.31	6.22
Total	100.49	100.42	100.45	100.70	99.15	100.91	100.22	99.64	100.89	100.19	99.81	100.65	100.29 ± 0.51	0.50

Mean ± SD from twelve replicates; [1]Coefficient of variation.

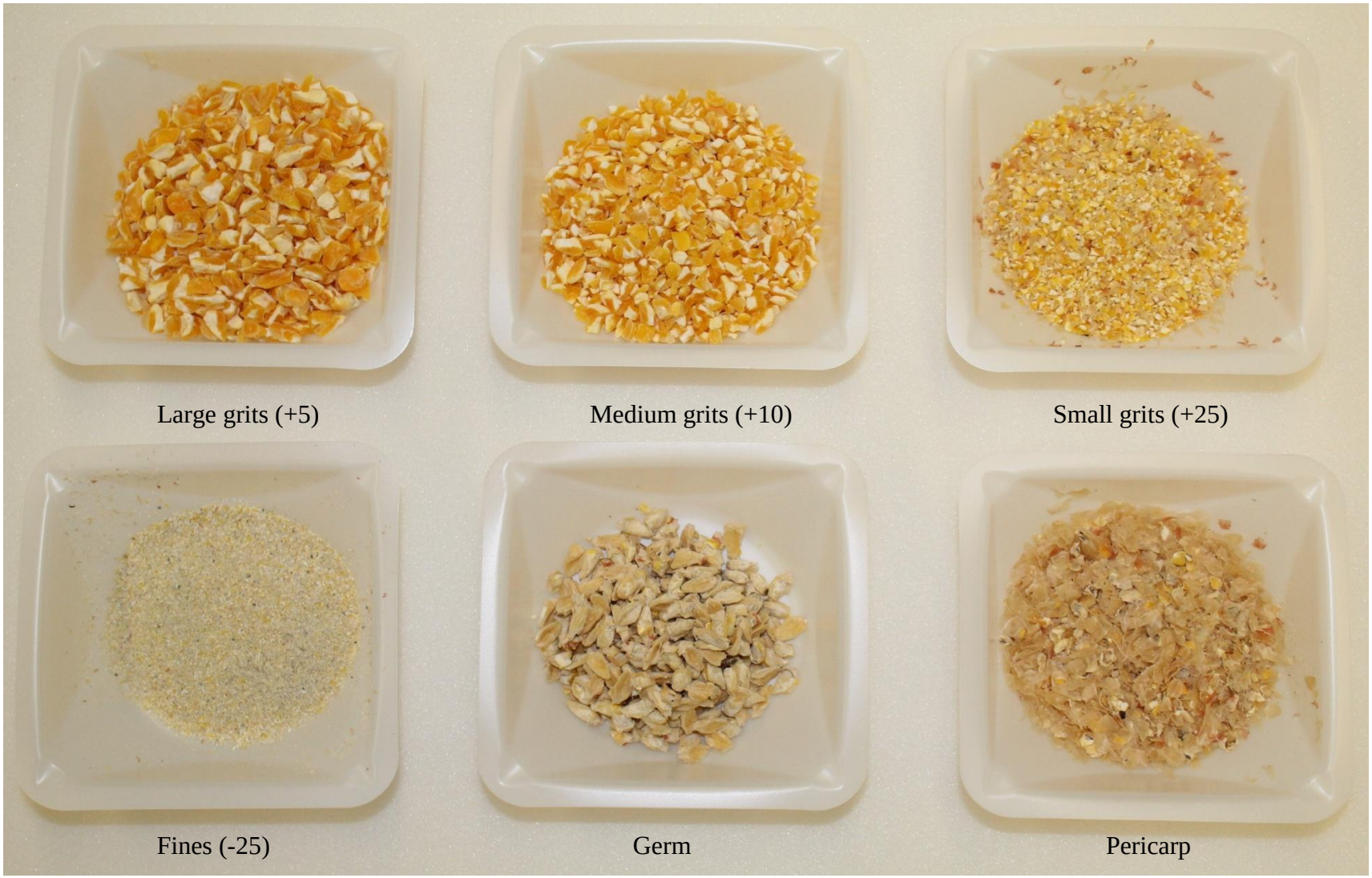


Fig. 6.2. Various coproducts from 100 g dry milling of Hard1 corn.

germ (13.21 to 11.51% db) and pericarp (5.59 to 4.59% db) also had low standard deviations at 0.44 and 0.31, respectively. COV for all coproducts were <10.0% verifying protocol robustness.

Rausch et al. (2009) dry milled 11 yellow corn hybrids and reported overall fraction yields for large grits, small grits, fines, germ and pericarp at 39.16, 25.25, 13.81, 14.29 and 6.83 g/100 g corn (db), respectively. Visual comparison of large grits, germ and pericarp from 100 g protocol, 1 kg protocol and commercial samples is included in Fig. 6.3. Smaller size of large grits and flattened germ can be observed for 1 kg protocol. In this study, one additional endosperm fraction, namely, medium grits (+10 stream) was recovered. Large grits yield in the current study corresponded to those reported in above studies while mean fines yield were lower at 4.38% (db). Lower fines yield can be attributed to the absence of roller milling step in 100 g protocol. Similarly, pericarp and germ yields also were lower in this study. These yield differences were due mainly to the hybrid/corn type effects. Overall, low standard deviations for various coproduct yields were observed using the 100 g protocol.

6.3.5. Dry milling characteristics of corn cultivars

Ten corn cultivars, including six yellow corn cultivars, three colored corn cultivars and one white corn, were processed using the 100 g protocol to ascertain dry milling characteristics (Table 6.5). Large grits yield was highest for white corn and lowest for red corn. The hard endosperm cultivars yielded higher proportion of large grits as compared to the colored corn. Medium grits (+10 stream) yield was highest for purple corn at 34.9% (db) and lowest for white corn (22.7% db). Colored corn yielded higher medium grits as compared to the hard endosperm corn. Colored corn cultivars had higher yields of small grits as compared to hard endosperm cultivars. The highest yield was for red corn and the lowest for Hard3 cultivar, the values being 16.4 and 7.7% (db), respectively. Fines yields were highest in red corn and high extractable starch cultivar (8.2% db) and lowest for blue corn (3.9% db). High amylose corn had the

TABLE 6.5. Dry milling characteristics of different corn cultivars (% db).

Cultivar	Large grits	Medium grits	Small grits	Fines	Germ	Pericarp	Total
Hard1	41.12 ± 1.32c	30.17 ± 0.64e	8.26 ± 0.14e	4.21 ± 0.30d	12.33 ± 0.38ab	4.06 ± 0.09f	100.17 ± 0.75ab
Hard2	44.21 ± 0.90b	23.70 ± 1.51fg	8.34 ± 0.35e	5.69 ± 0.54c	12.59 ± 0.73a	5.87 ± 0.20e	100.39 ± 0.15ab
Hard3	46.97 ± 1.01a	24.97 ± 1.01f	7.75 ± 0.06ef	4.19 ± 0.23d	11.24 ± 0.06bc	5.21 ± 0.06e	100.33 ± 0.52ab
HE	25.23 ± 1.11e	34.85 ± 0.60ab	12.67 ± 0.11c	8.16 ± 0.26a	11.96 ± 0.62ab	7.88 ± 0.48d	100.75 ± 0.13ab
HA	26.38 ± 0.78e	33.51 ± 0.86bc	11.68 ± 0.22d	5.61 ± 0.07c	12.94 ± 0.67a	11.00 ± 0.49a	101.12 ± 0.51a
Waxy	31.81 ± 1.33d	31.29 ± 1.04de	11.69 ± 0.12d	6.83 ± 0.30b	9.67 ± 0.12d	9.10 ± 0.20c	100.60 ± 0.98ab
Purple	21.34 ± 0.08f	34.89 ± 1.02ab	14.72 ± 0.66b	7.17 ± 0.26b	9.68 ± 0.45d	13.21 ± 0.35a	101.01 ± 0.35a
Blue	31.88 ± 1.43d	32.71 ± 1.39cd	11.78 ± 0.34d	3.91 ± 0.81d	10.28 ± 0.67cd	9.55 ± 0.09c	100.12 ± 0.51ab
Red	20.87 ± 0.37f	35.77 ± 0.42a	16.40 ± 0.33a	8.16 ± 0.12a	9.60 ± 0.63d	8.96 ± 0.56c	99.78 ± 0.64b
White	48.59 ± 0.89a	22.67 ± 1.23g	7.60 ± 0.05ef	5.78 ± 0.11c	7.72 ± 0.44e	7.82 ± 0.39d	100.19 ± 0.44ab

Mean ± SD from three replicates; Means followed by the same letter in a column are not different ($p < 0.05$).
Hard1, Hard2 and Hard3: hard endosperm cultivars; HE: high extractable starch corn; HA: high amylose corn; Waxy: waxy corn; Purple, Blue and Red: colored corn cultivars; White: white corn.

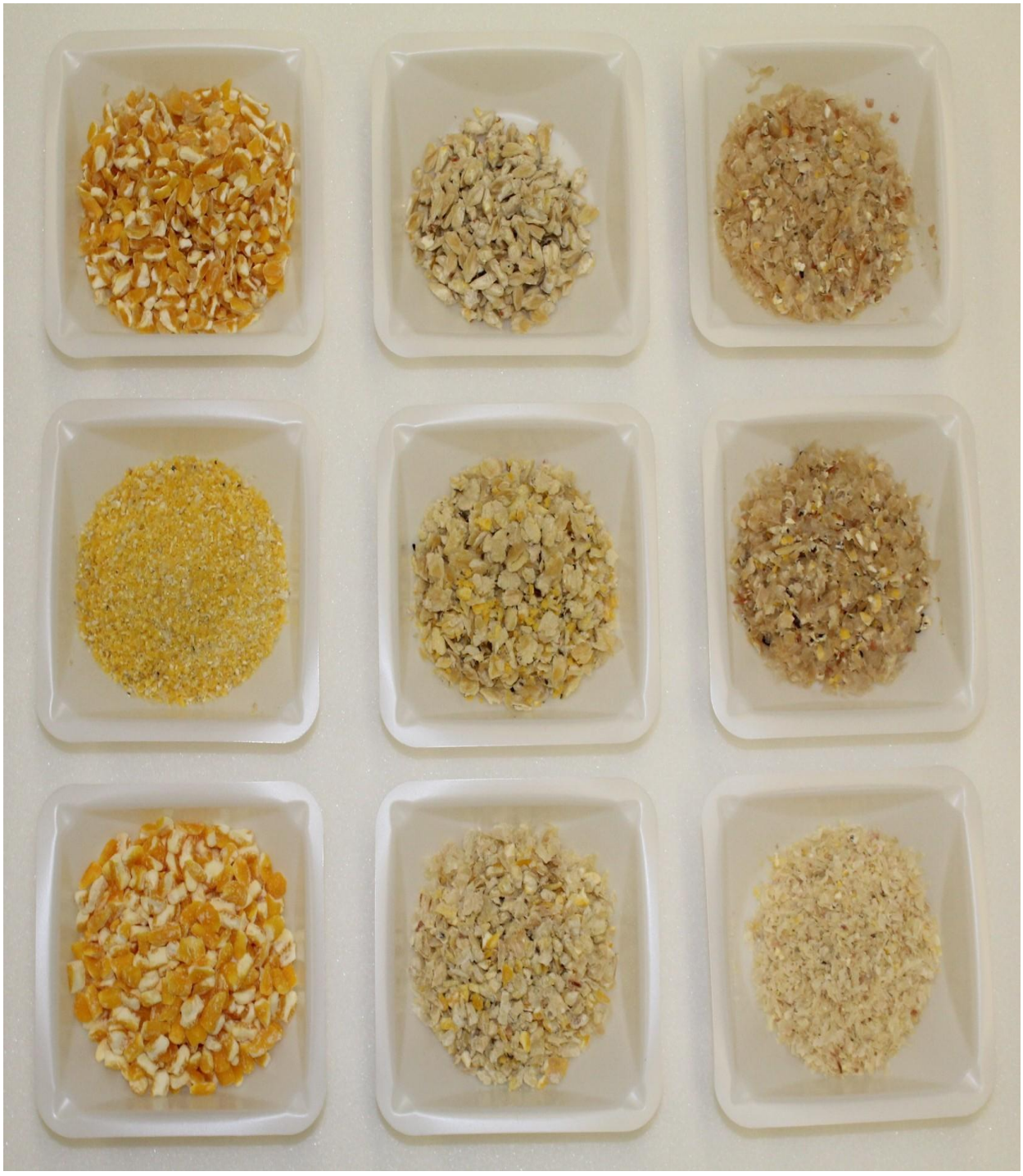


Fig. 6.3. Comparison of large grits, germ and pericarp (left to right) from 100 g dry milling protocol (top), 1 kg protocol (middle) and commercial samples (bottom).

highest germ yield (12.9% db) while it was lowest for white corn (7.7% db). Pericarp yield was highest for purple corn and lowest for Hard1 cultivar, the values being 13.2 and 4.1% (db), respectively. Hard endosperm cultivars of corn had low pericarp yields as compared to the colored corn cultivars. Mean total coproduct yields were high for all corn cultivars, indicative of the robustness of 100 g protocol.

6.3.6. Compositional analysis of coproducts from different corn cultivars

Coproduct fractions from corn were analyzed for crude protein, crude oil and neutral detergent fiber content. For comparison purposes, commercial coproduct samples also were analyzed (Table 6.6). For large grits, highest protein content was in blue corn and lowest in commercial grits. Crude oil content was >3.0% for large grits from purple, blue and high amylase corn and <1.0% for the hard endosperm cultivars. Commercial large grits contained minimum crude oil (0.27% db). Duensing et al. (2003) reported the fat content of commercial flaking grits to be <0.8%. In this study, the fat content of large grits from hard endosperm cultivars was <0.87%. The fat content of lab scale large grits varied from 1.1 to 1.6% (Peplinski et al. 1984) and 1.0 to 1.9% (Rausch et al. 2009). Neutral detergent fiber content was highest in large grits from high amylose corn and lowest in commercial grits, the values being 17.94 and 0.89% (db), respectively.

In case of medium grits, blue corn had the highest crude protein content while waxy corn had the lowest, the values being 10.17 and 6.74% (db), respectively. Highest oil content was observed in high amylose corn (2.99% db) while Hard3 cultivar had the lowest (0.78% db), the value for commercial sample was 1.35% (db). The variation of crude oil content in medium grits from hard endosperm cultivars (0.78 to 1.29% db) was lower than from color corn cultivars (1.82

TABLE 6.6. Compositional analysis of coproducts and comparison with commercial samples (% db).

		Corn cultivars										
Fractions	**Analyte**	**Hard1**	**Hard2**	**Hard3**	**HE**	**HA**	**Waxy**	**Purple**	**Blue**	**Red**	**White**	**Plant**[1]
Large grits	CP	7.02e	8.16d	7.13e	7.10e	9.51c	6.86e	10.24b	10.78a	8.38d	9.46c	6.12f
	Oil	0.87f	0.76f	0.55g	0.80f	3.09c	1.13e	3.31b	3.69a	1.24e	1.76d	0.27h
	NDF	4.25e	4.11e	4.04e	3.73e	17.94a	4.95d	8.96b	7.29c	5.09d	4.00e	0.89f
Medium grits	CP	7.30d	8.34c	7.53d	6.74e	9.15b	6.65e	9.89a	10.17a	8.48c	9.12b	6.86e
	Oil	1.07f	1.29e	0.78g	0.81g	2.99a	1.00f	2.05c	2.38b	1.82d	1.98c	1.35e
	NDF	3.45de	3.54cde	3.55cde	2.67f	17.03a	3.87cd	4.84b	4.07cd	4.15c	3.00ef	2.03g
Small grits	CP	8.71e	9.81c	9.27d	8.31e	9.52cd	7.71f	9.26d	10.27b	9.36d	11.65a	6.62g
	Oil	1.59f	3.14a	2.09d	0.89g	3.19a	1.53f	1.91e	2.32c	2.87b	3.13a	0.69h
	NDF	11.18c	11.10c	9.78d	6.02f	23.47a	7.91e	6.66f	8.48e	6.30f	12.07b	1.45g
Fines	CP	6.43g	7.11d	7.23c	5.84i	7.55b	5.34j	6.82f	6.97e	6.38g	8.10a	6.17h
	Oil	1.00cde	1.23bc	1.04cd	0.76ef	2.16a	1.10cd	0.77ef	0.73f	1.48b	1.24bc	0.89def
	NDF	6.45b	6.14bc	5.79c	3.19f	20.09a	4.83d	3.94e	4.99d	3.29f	6.02c	1.89g
Germ	CP	18.37b	17.65c	17.68c	18.59b	15.28f	18.59b	43.01a	14.53b	16.10e	16.87d	14.46g
	Oil	26.68f	26.75f	28.15d	26.23g	29.16c	24.66h	30.24b	30.04b	33.54a	27.44e	18.12i
	NDF	21.06e	20.86e	28.15b	25.39c	22.31d	21.65de	17.78f	17.74f	21.43de	32.35a	13.82g
Pericarp	CP	6.92d	16.87a	7.68c	8.08c	7.00d	6.87d	8.90b	8.73b	8.68b	7.88c	7.06d
	Oil	2.02e	4.97a	1.99e	1.53f	3.63cd	1.76ef	3.74c	4.31b	4.28b	3.28d	3.78c
	NDF	46.38d	40.58f	35.76g	41.25ef	58.04a	53.59b	35.77g	41.15f	45.07d	48.32c	42.55e

[1]Coproduct samples from a commercial dry milling plant. CP: crude protein, Oil: crude oil, NDF: neutral detergent fiber.
Mean of two replicates; Means followed by the same letter in a row are not different ($p < 0.05$).

to 2.38% db). Highest neutral detergent fiber content was observed in high amylose corn while lowest was in commercial samples, the values being 17.03 and 2.03% (db), respectively.

For small grits, highest crude protein content was determined in white corn (11.65% db) while the lowest was in commercial sample (6.62% db). Crude oil content values varied from 3.19 to 0.69% (db) with high amylose corn having the maximum and commercial sample the minimum value. Highest neutral detergent fiber (23.47% db) was observed in high amylose corn while lowest was detected in commercial sample (1.45% db). Crude protein content in fines ranged from 8.10 to 5.34% (db) with white corn having the highest and waxy corn the lowest values. Fines from high amylose corn had highest crude oil (2.16% db) and neutral detergent fiber (17.01% db) concentrations. Lowest crude oil content was in blue corn (0.73% db) and lowest neutral detergent fiber (1.89% db) was in fines sample from industry sample.

Crude protein content in germ varied between 43.01 to 14.46% (db) with purple corn germ having the maximum and commercial sample having the minimum values. Highest crude oil content was detected in red corn germ (33.54% db) while the lowest was in the commercial germ sample (18.12% db). Colored corn germ samples had higher crude oil contents compared to hard endosperm cultivars. White corn germ had highest neural detergent fiber (32.35% db) while commercial germ sample had the minimum amount (13.82% db). For pericarp, highest neutral detergent fiber was quantified in high amylose corn (50.04% db) while the minimum value was detected in Hard3 cultivar (35.76% db). All commercial coproduct samples had lower crude oil contents compared to other samples in this study.

6.4. Conclusions

A 100 g dry milling procedure for estimation of coproduct yield and composition was developed. The protocol required smaller amount of samples and estimated coproduct yields with low standard deviations with respect to the means for different hybrids. Unlike a 1 kg protocol, no roller milling step was involved; as a result true sized large grits were recovered and fines yield were lower. Four endosperm fractions (large grits, medium grits, small grits and fines) were recovered. Crude oil contents of large and medium grits for hard endosperm cultivars of corn ranged from 1.29 to 0.55% (db), respectively and were higher than commercial samples but comparable to the other lab scale studies. Colored corn grit samples had higher crude oil content as compared to the hard endosperm cultivars (3.69 to 1.24% db). Colored corn grits also had higher crude protein content as compared to the hard endosperm cultivars. Hybrid effects were responsible for variations in coproduct yields and compositions for various corn cultivars and may affect commercial scale processing efficiencies. Due to the smaller amount of required samples and shorter processing times, this protocol should be useful in estimating dry milling characteristics of different corn hybrids. True sized large grits are recovered, allowing better assessment of a hybrid's suitability for dry milling process.

Chapter 7. Conclusions and recommendations

7.1. Conclusions

Due to potential health concerns associated with the ingestion of synthetic dyes and an expanding natural food color market, we focused on exploring the potential of using colored corn as a source of anthocyanins. Many researchers have suggested use of anthocyanins as a potential replacement of FD&C Red 40 dye for food and beverages. Our main research hypothesis was that colored corn can be utilized for anthocyanin recovery and the process could be tailored in a way such that processed coproducts could be utilized further. This would give colored corn an advantage over conventional sources of anthocyanins. With respect to the specific objectives outlined in the beginning, major conclusions are summarized below:

1. In wet milling process, starch yields for purple and blue corn were 63.4 and 61.5% (db), respectively, as compared to conventional yellow dent corn 70.1% (db). Mean starch whiteness values for yellow and blue corn, 90.0 and 90.1, respectively, were higher as compared to purple corn starch (80.6). In dry milling, large grit yields for purple and blue corn were 21.8 and 24.4% (db), respectively, implying dominant softer endosperm. In dry grind process, final ethanol concentrations after 72 hr fermentation for purple, blue and yellow dent corn were 14.5, 14.4 and 17.2% (v/v), respectively.
2. Concentrations of pigments inside corn kernel depend upon corn genetics. Purple corn contained 13 times more anthocyanins as compared to blue corn. For purple corn, maximum amounts were in steepwater from wet milling and pericarp from dry milling. Microscopic imaging revealed maximum concentration of pigments in the pericarp. In case of blue corn, maximum anthocyanins were in gluten slurry from wet milling and small grits from dry milling. Unlike in purple corn, pigments were concentrated in

aleurone layer. Corn fractionation generated coproducts with disproportionately higher concentration of pigments which could be harnessed to source anthocyanins economically.

3. Addition of SO_2 in the treatments facilitated greater extraction of anthocyanins; it also had a bleaching effect on the extract color. Chroma was correlated with the concentration of anthocyanins and tannins. In steeping treatments with only water, condensed form was the dominant anthocyanins form while in all other treatments, cyanidin-3-glucoside and its acylated forms were most abundant. Purple corn pericarp, recovered using dry milling process, contained 23 g anthocyanins/kg pericarp making it one of the richest sources of anthocyanins on a w/w basis.
4. Smaller samples were required for the procedure while coproduct yields for different hybrids were estimated with low standard deviations with respect to means. Although higher than commercial grits, this value was comparable with other lab scale studies. Hybrid effects were responsible for variations in coproduct yields and compositions for various corn cultivars.
5. It was found that purple corn, on account of its greater anthocyanin content and their concentration in pericarp, will be a good candidate for anthocyanin recovery. During wet milling of purple corn, steepwater will contain maximum pigments and can be spray dried for their recovery. Another approach will involve recovery of anthocyanin rich pericarp at the front end using dry milling. Pericarp can be selectively processed for anthocyanin recovery while remaining endosperm can be dry milled or converted into ethanol by dry grind process.

7.2. Recommendations for future work

Investigation of following issues will assist researchers and industry personnel in effectively applying knowledge gained from this work:

1. Milling characteristics and potential of more colored corn cultivars should be evaluated. Recovery of diverse anthocyanin forms will help food industry in coloring solutions for broad range of products.
2. Optimization of pericarp recovery from purple corn is required. During milling, some purple corn pericarp breaks down, gets distributed in other coproduct streams and hard to recover.
3. Pericarp steeping parameters like temperature, amount of SO_2 and duration may be optimized for maximum anthocyanin recovery.
4. Techno-economic feasibility analysis of replacing yellow dent corn by colored corn in conventional corn fractionation industry is required. These models using process modeling software will help in eventual implementation of the concept in industry. Models will help in identifying inputs and process parameters that will be critical in ensuring efficient anthocyanin recovery from colored corn.

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Appendix A. **Results of 500 g experiments using 1 kg protocol**

Table A.1. Dry milling coproduct yields from colored corn hybrids (% db).

	50x249		50x281		P22x249		15x249
	1	2	1	2	1	2	1
Large grits	18.48	18.89	30.60	29.07	29.66	29.32	23.79
Small grits	23.84	28.16	24.33	25.51	30.75	32.95	32.18
Fines	42.01	35.18	29.95	27.15	27.18	26.46	29.73
Germ	8.48	9.80	7.63	9.06	7.04	7.32	9.18
Pericarp	7.99	9.66	6.35	7.96	6.37	6.56	6.75
Total	100.79	101.70	98.85	98.74	101.00	102.61	101.62

Appendix B. **Process flow for quantification of anthocyanins and polyphenols**

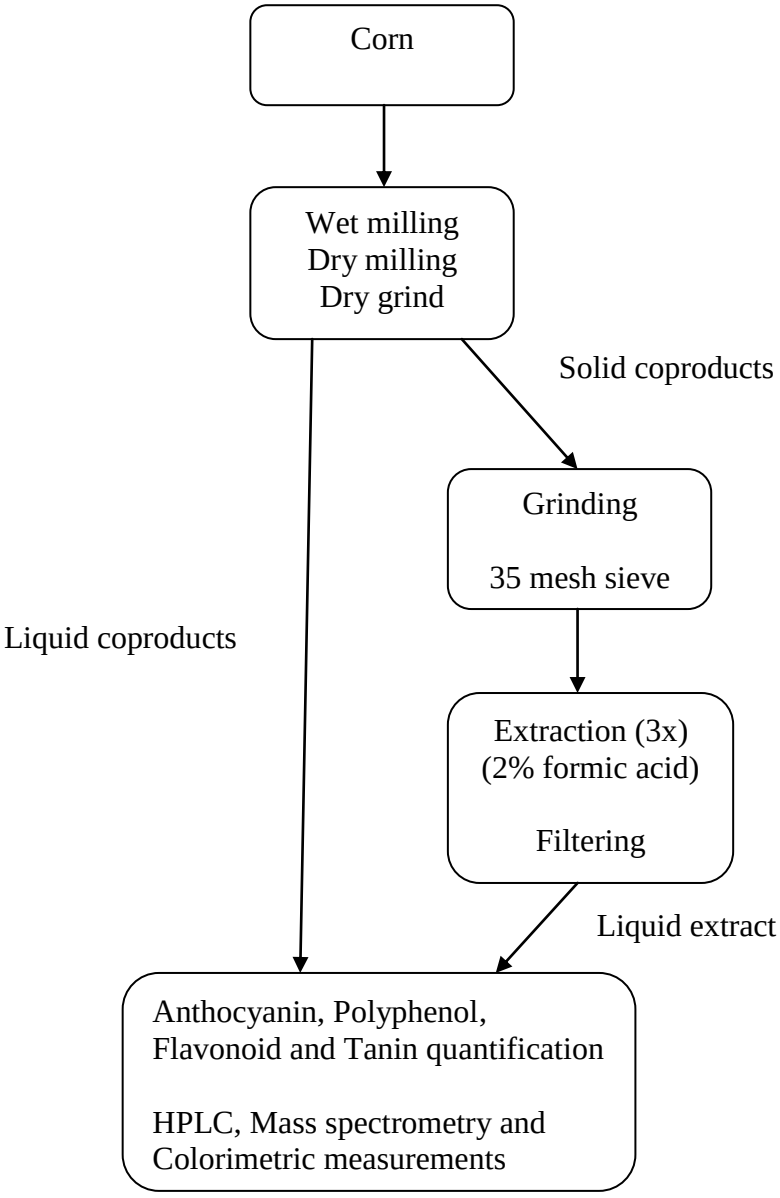


Fig. B1. Flow chart for coproduct anthocyanin and polyphenol quantification.

Appendix C: Coproducts from wet milling, dry milling and dry grind processes

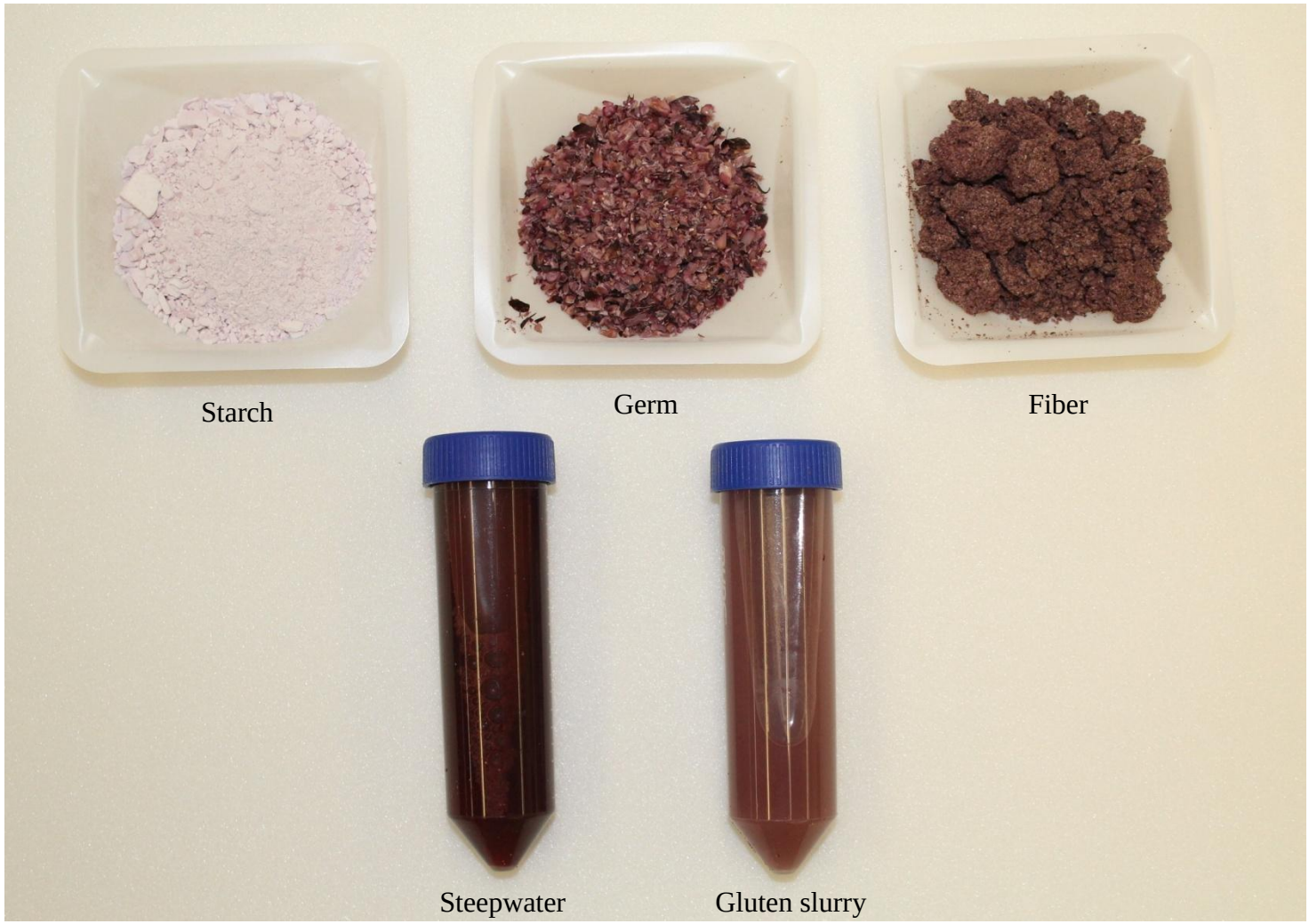


Fig. C.1. Coproducts from purple corn wet milling.

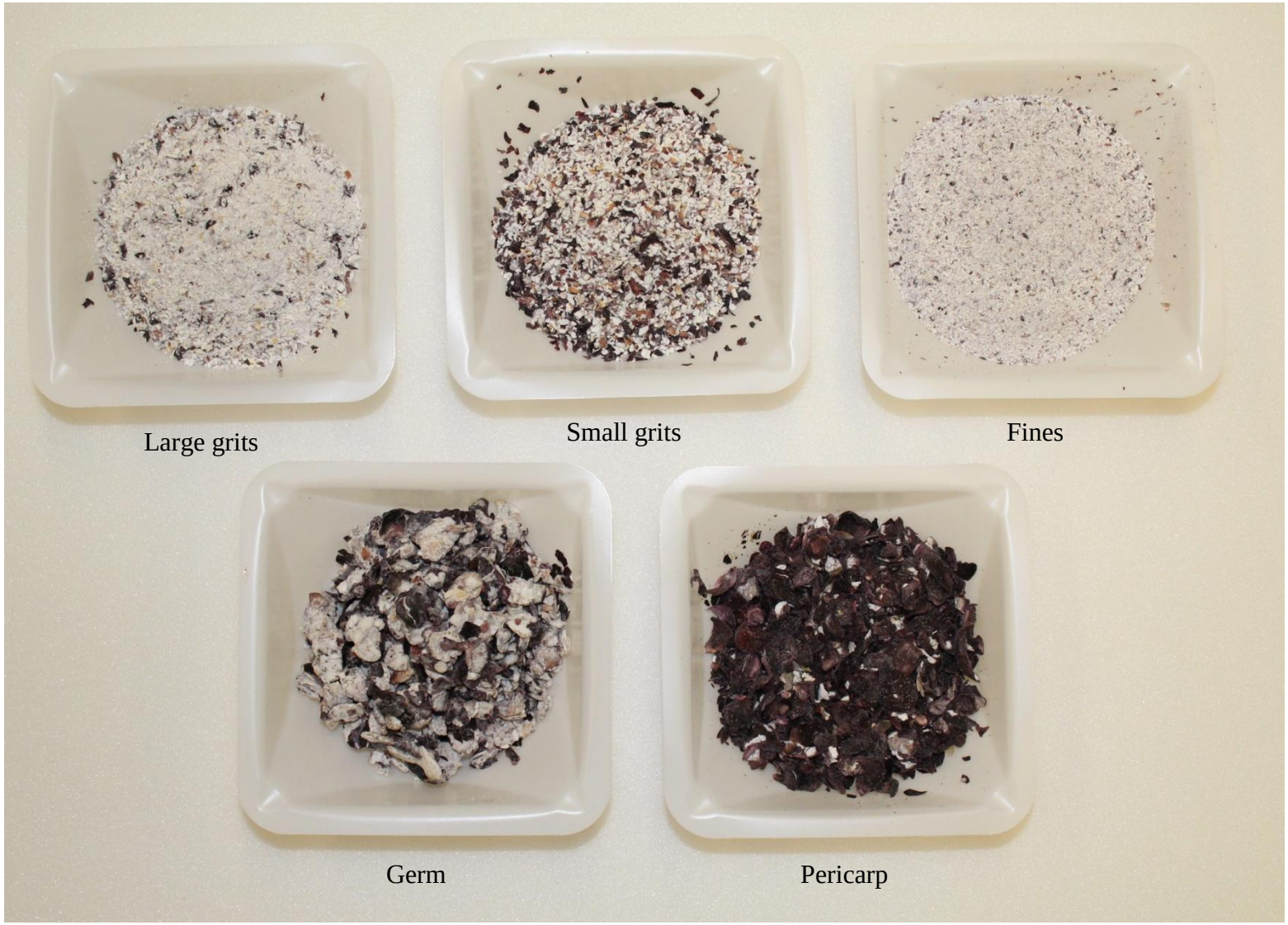


Fig. C.2. Coproducts from purple corn dry milling.

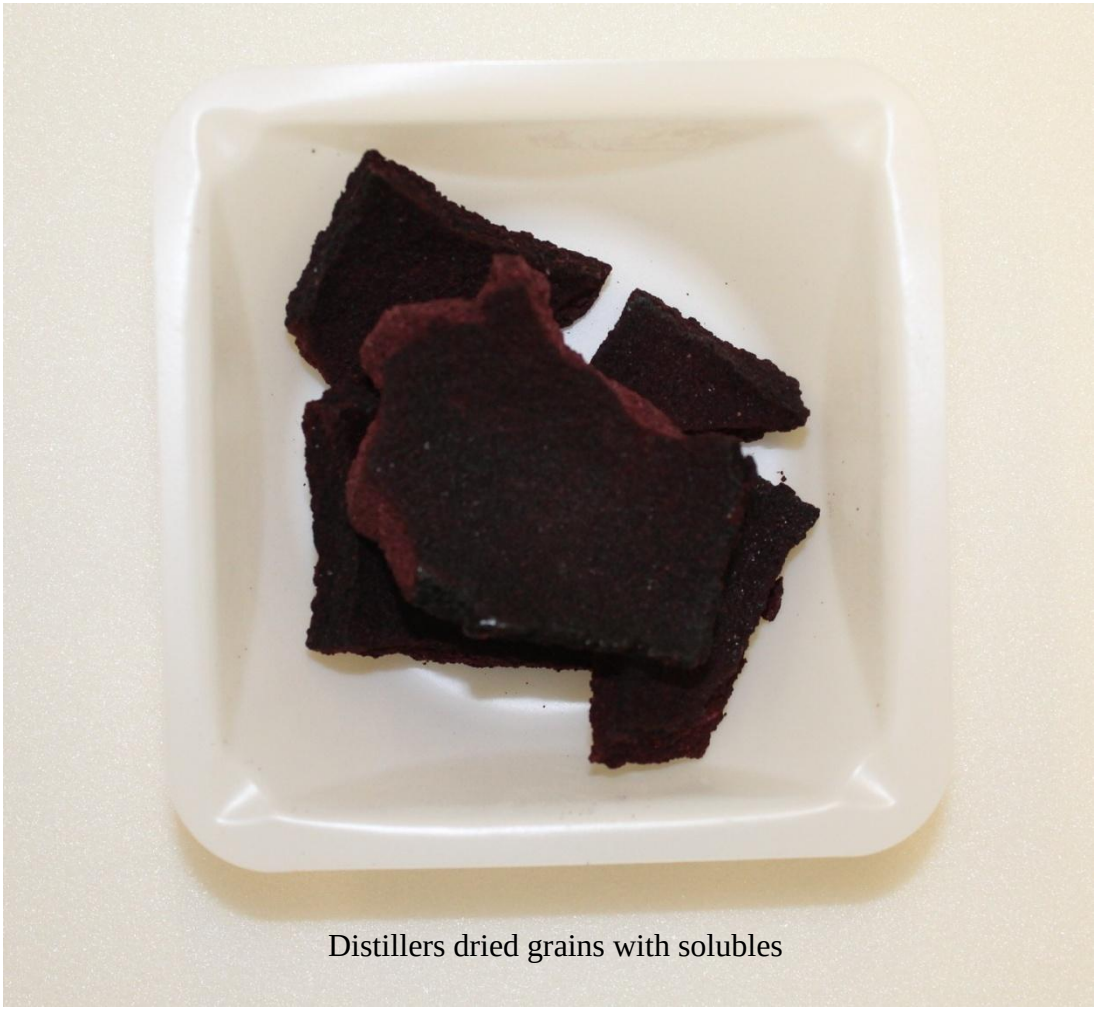


Fig. C.3. Coproduct from purple corn dry grind process.

Appendix D: Wet milling, dry milling and dry grind process flow

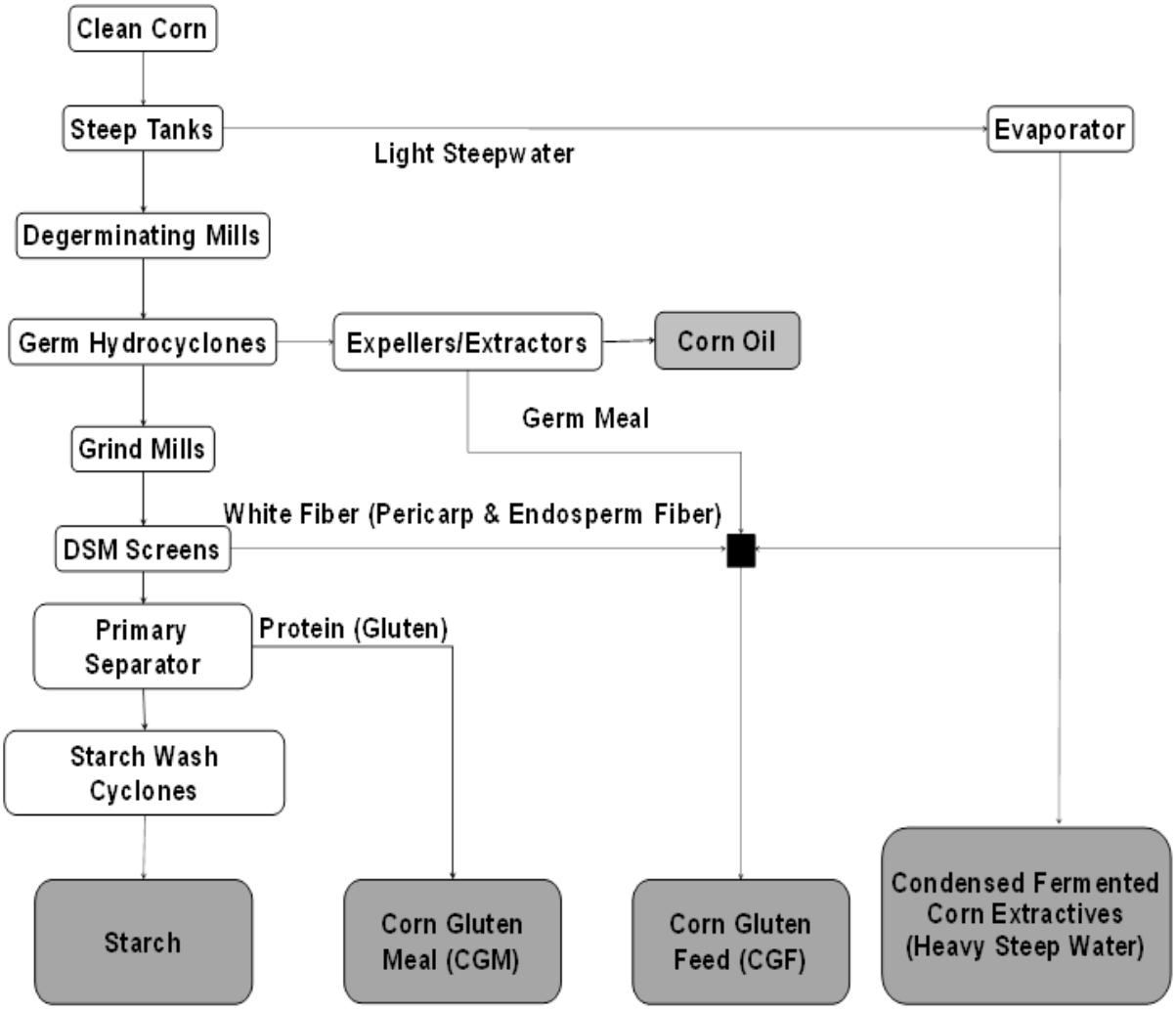


Fig. D.1. Wet milling process flow.

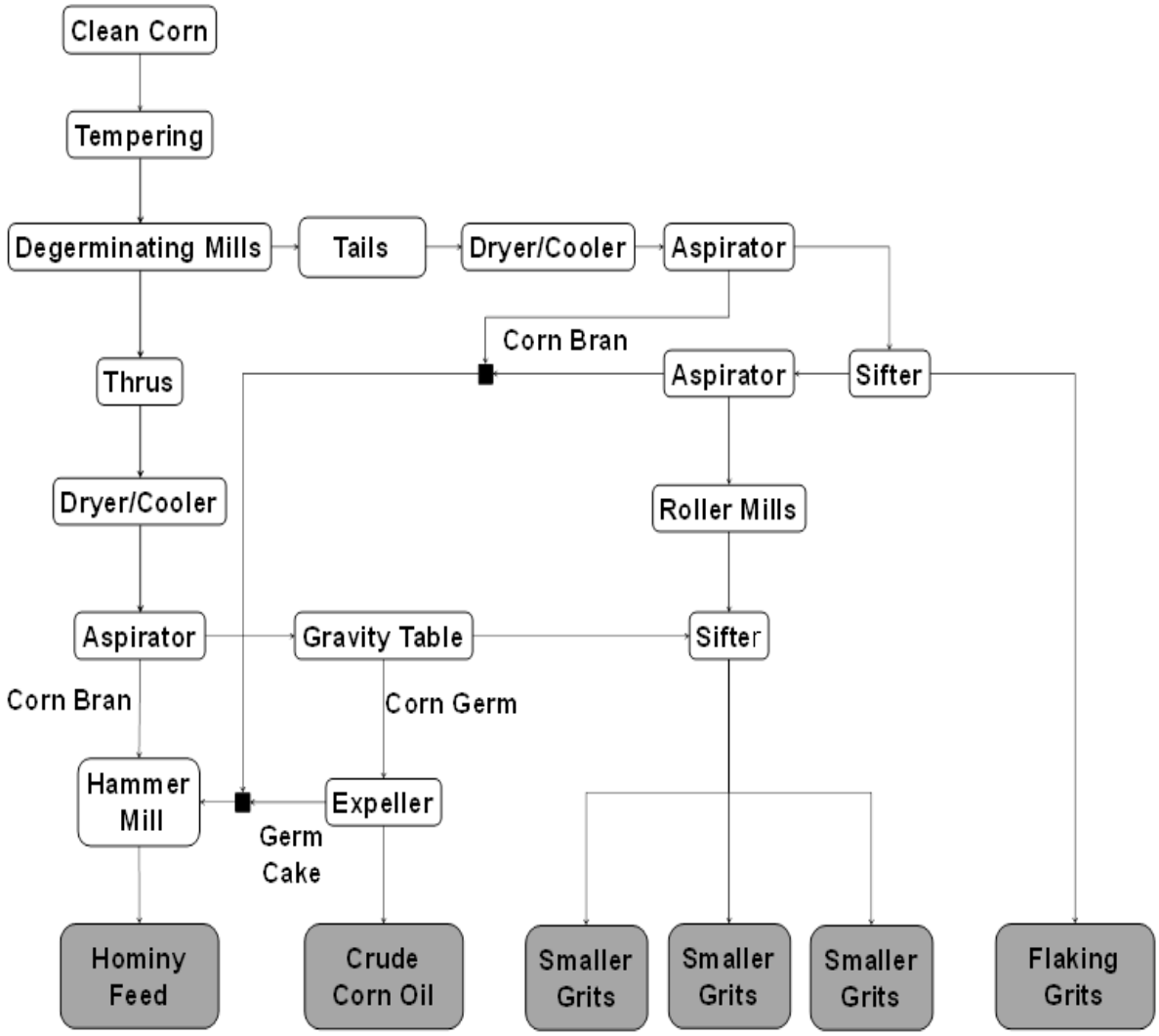


Fig. D.2. Dry milling process flow.

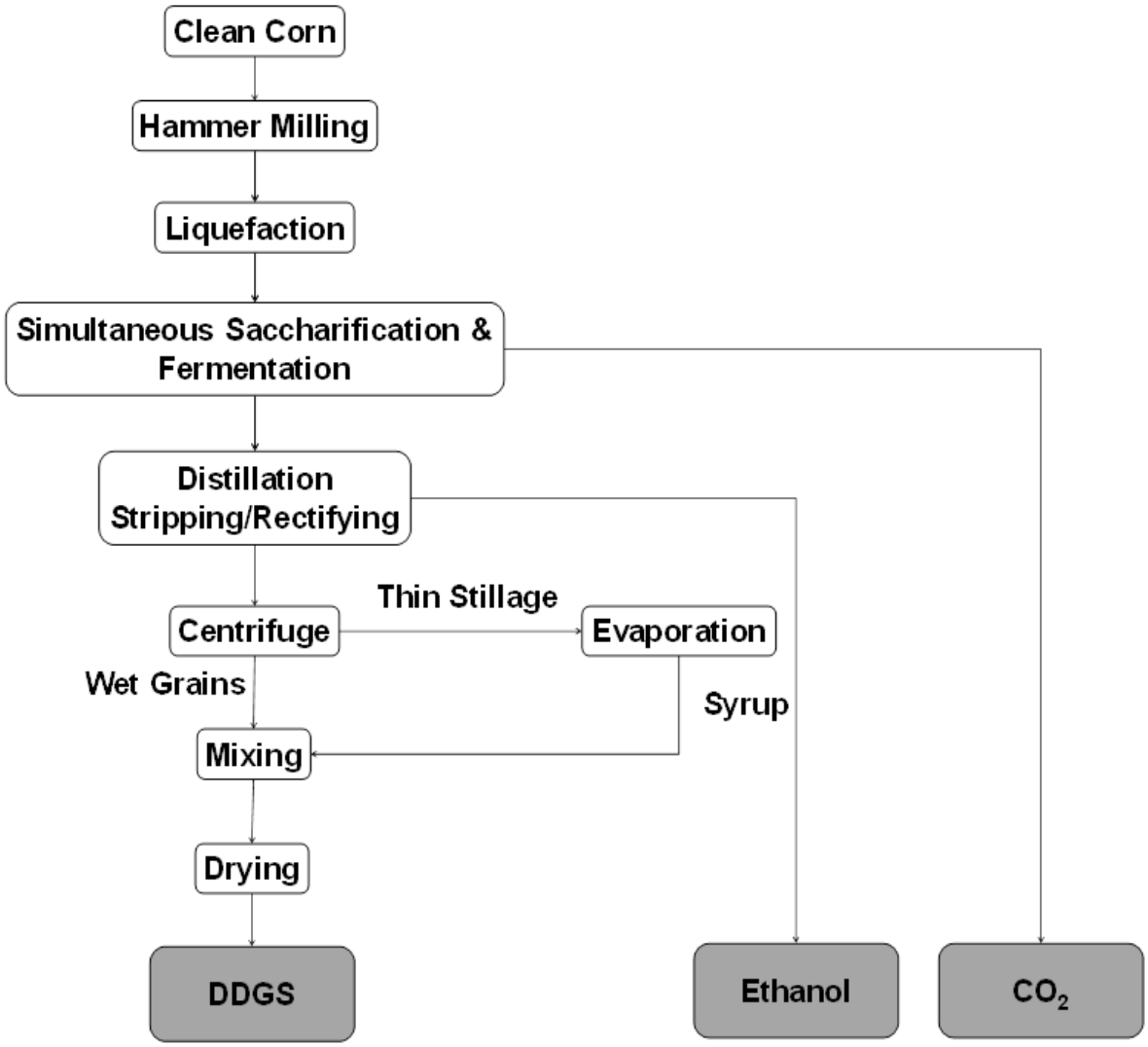
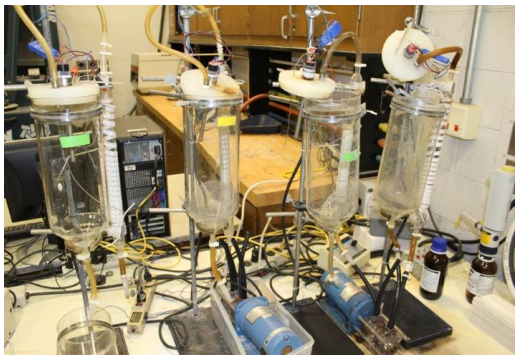


Fig. D.3. Dry grind process flow.

Appendix E: Equipment for lab scale wet milling, dry milling and dry grind processes

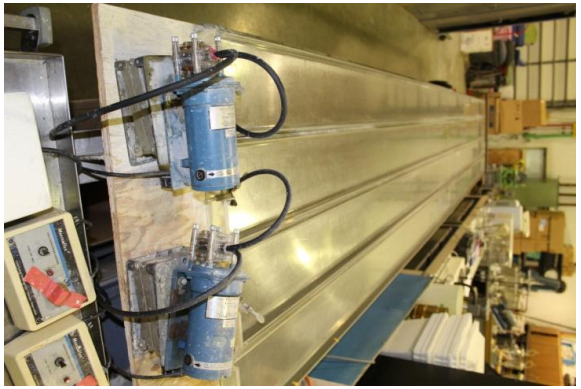


Steeping vessels

Blender for first grind

Plate mill for second grind

Vibratory shaker (270 mesh screen)



Gravity table

Fig. E.1. Equipment used for lab scale wet milling of corn.

Electronic moisture meter

Tempering vessels

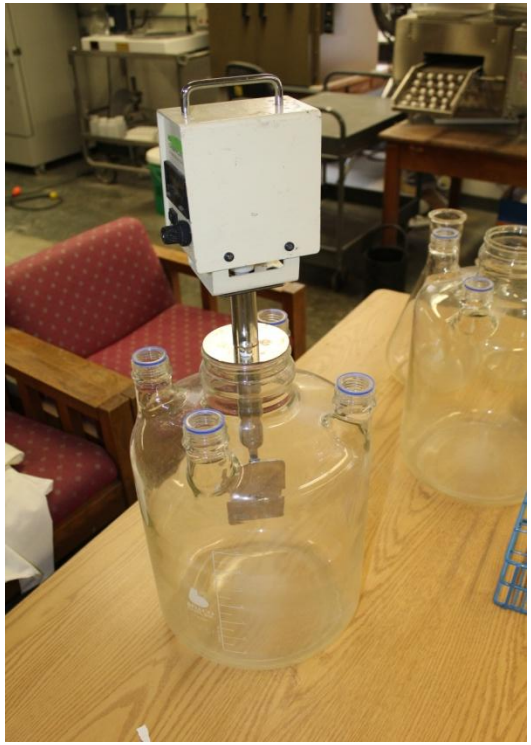
Roller mill

Drum degerminator

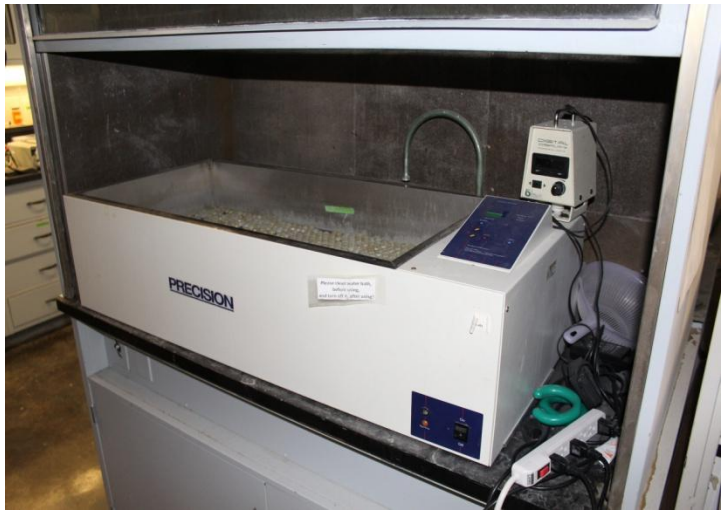
Box sifter

Aspirator

Fig. E.2. Equipment used for lab scale dry milling of corn.



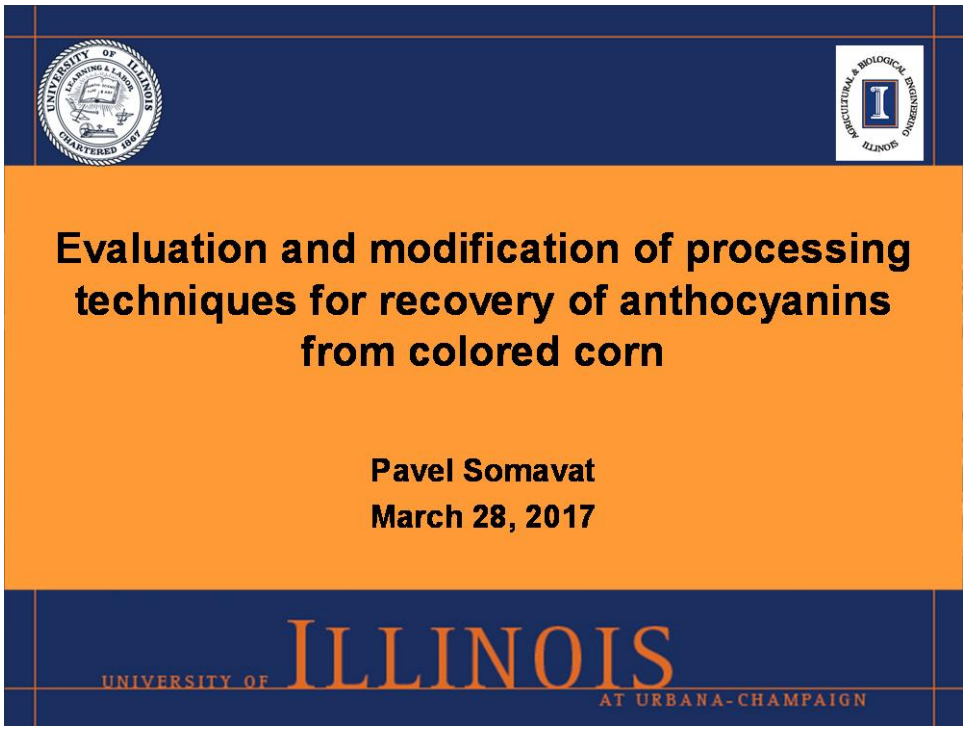
Fermentation flask with agitator

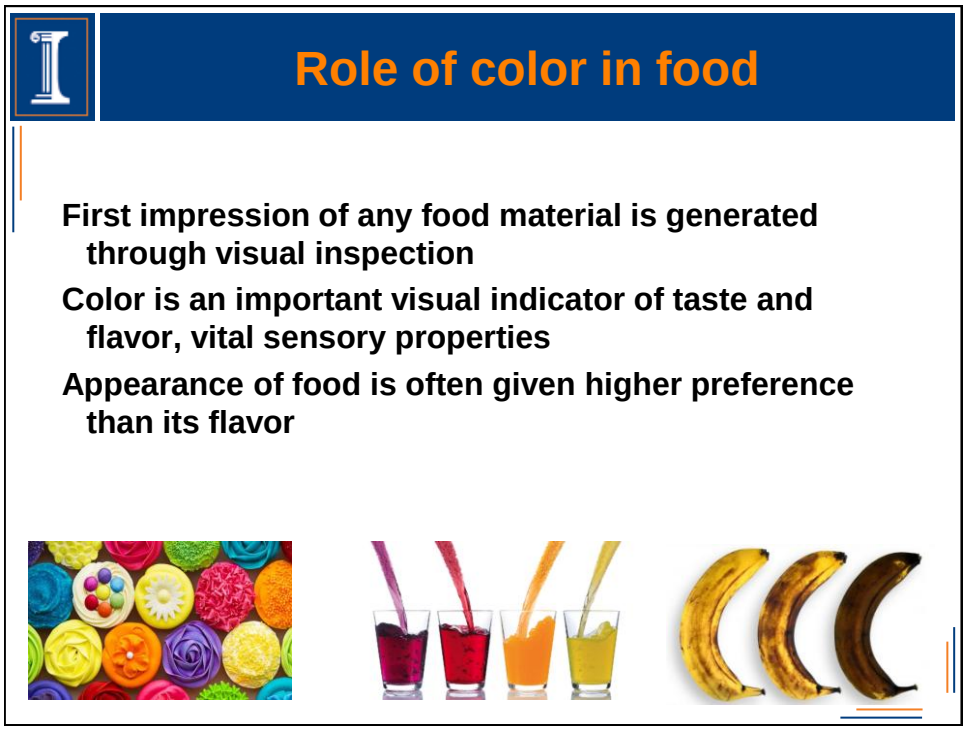


Water bath for liquefaction and fermentation

Fig. E.3. Equipment used for lab scale dry grind processing of corn.

Appendix F: Presentation slides





Artificial food colorants

- Preferred due to lower costs, higher stability and a wider palette
- Feingold (1975) reported that food additives cause attention-deficit/hyperactivity disorder (ADHD)
- Azo dyes may cause genotoxicity, neurotoxicity and/or carcinogenicity
- Consumption has increased five fold since 1950's to 67 mg per person per day

Natural colors

- General move towards "natural ingredients" and health concerns associated with synthetic dyes
- Humans have been eating carotenoids, anthocyanins, betalains, curcumin and chlorophylls
- Expensive, less stable and difficult to use due to varying solubility

Anthocyanins (ACNs) as food color

- ACNs are vacuolar pigments which impart red, purple, blue and black colors to the plant matter
- Can be used as a replacement of FD&C Red 40 dye; may also deliver health benefits
- Sourced from purple colored fruits and vegetables such as dark grapes, dark carrots and red cabbage
- Various approaches used include solvent based extraction and juice concentration

However, due to greater inputs in terms of plant capacity, chemicals, material processing and lower economic value of the processing residue, anthocyanin recovery remains uneconomical

Research hypothesis

Colored corn processing could be optimized for anthocyanin recovery and subsequent residue utilization, yielding significant processing advantages

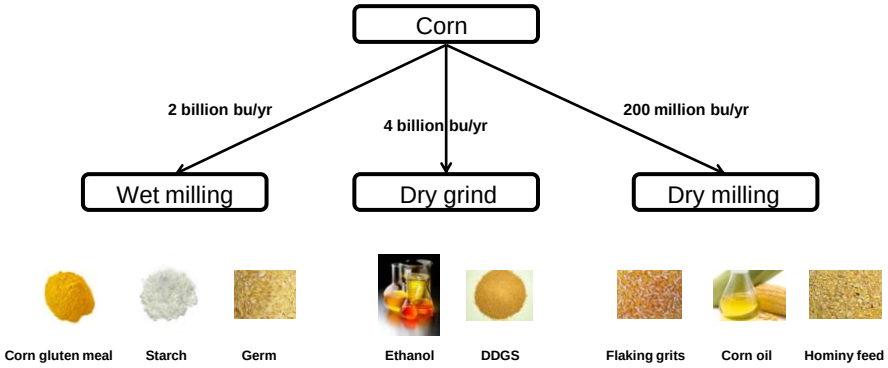
Objectives

1. To compare coproduct yields of colored corns with yellow dent corn for various milling processes
2. To study the distribution and yield of anthocyanins in various corn coproducts
3. Optimization of anthocyanin recovery from purple corn pericarp using wet milling chemicals
4. Development of a 100 g dry milling protocol for the measurement of coproduct yield and composition

Objective 1

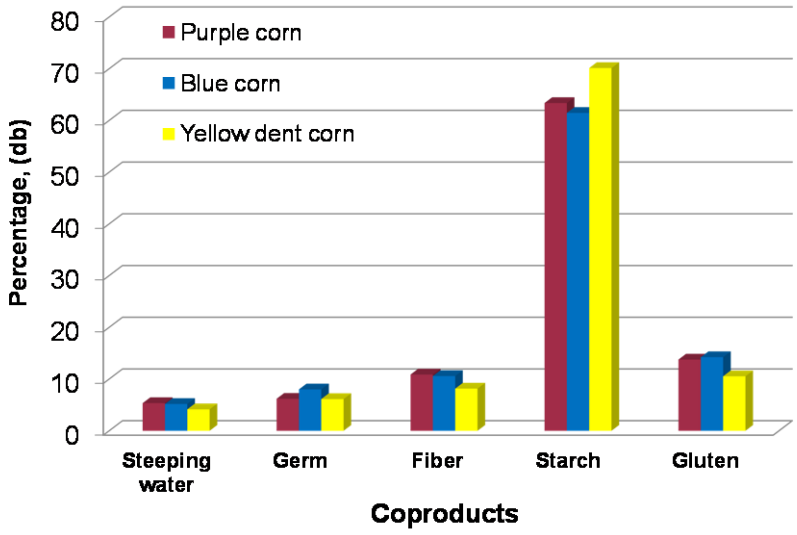
To compare coproduct yields of colored corn against yellow dent corn for wet milling, dry milling and dry grind processes

Corn processing industry

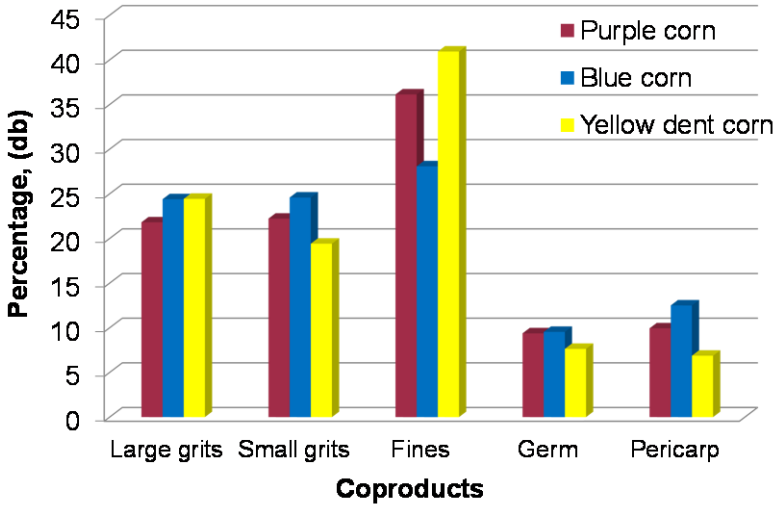


One kg lab scale procedures were used to test colored corn performance

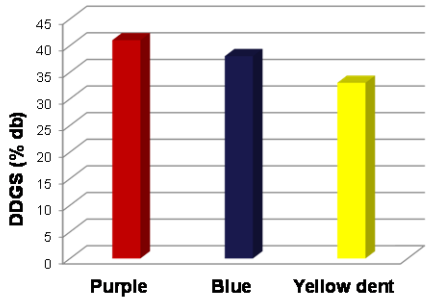
Wet milling characteristics

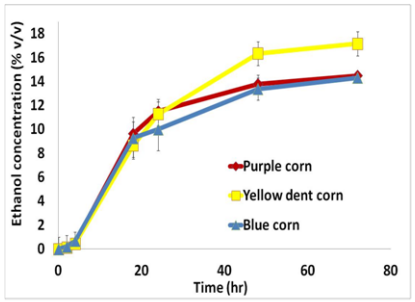


Dry milling characteristics



Dry grind characteristics





Conclusions

- In wet milling, starch yields were 7-9% higher for yellow dent corn
- Low large grit yields for all three corn in dry milling
- In dry grind process, high ethanol yields for yellow corn (2.7% v/v); DDGS yield was highest for purple
- Purple and blue corn can be used, albeit with some yield differences

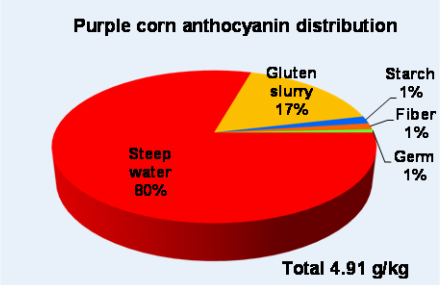
Objective 2

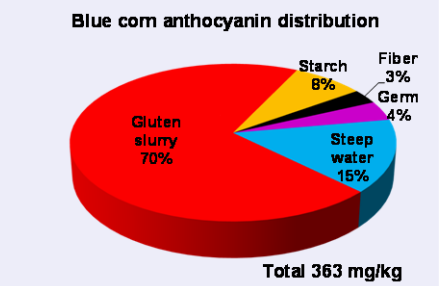
To study distribution and yield of purple and blue corn anthocyanins in milling coproducts from three processes

Materials and methods

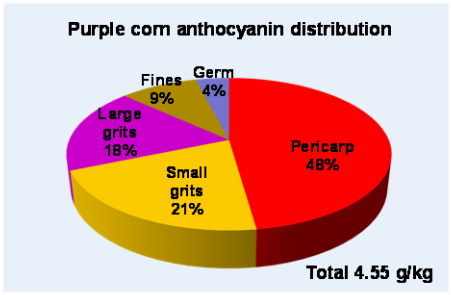
- Solid coproducts were ground and material (0.5 g) was extracted in 2% aqueous formic acid for 2 hr
- Suspension was filtered and filterate analyzed for anthocyanins and HPLC/MS-MS analysis
- Anthocyanins were measured using pH differential method
- HPLC analysis and mass spectrometry for anthocyanin identification and profiling using standard methods

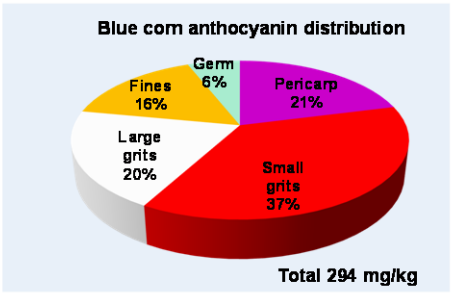
ACN distribution in wet milling



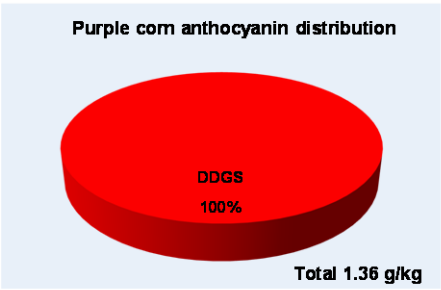


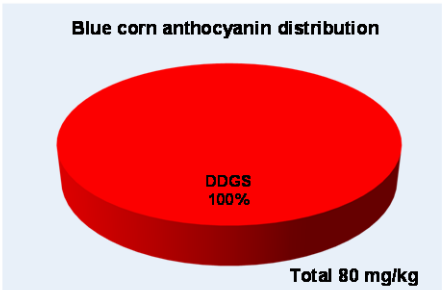
ACN distribution in dry milling



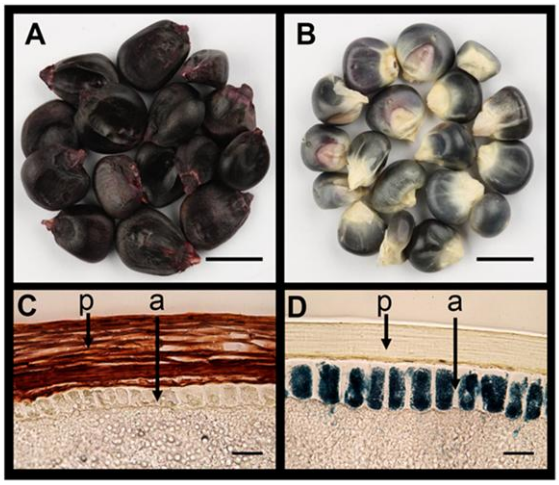


ACN distribution in dry grind





Bright field images



A: Purple corn; B: Blue corn

p: Pericarp layer; a: aleurone layer

Conclusions

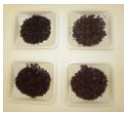
- **Anthocyanin concentration in purple corn (4.7 g/kg) was much higher than blue corn (0.4 g/kg)**
- **Location of pigments inside corn kernel depend upon corn genetics**
- **Corn processing can generate coproducts with disproportionately high ACN concentrations**
- **Maximum ACNs (80%) were quantified in wet milling in steepwater**

Objective 3

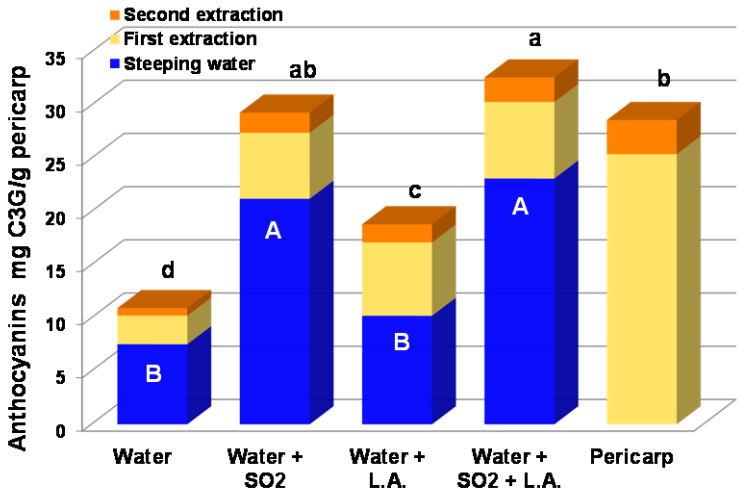
Optimization of anthocyanin recovery from purple corn pericarp using wet milling chemicals

Materials and methods

- **Purple corn pericarp (10 g) steeped in four different combinations and incubated for 24 h at 52°C**
  - **i) 125 mL water**
  - **ii) 125 mL water and 0.27 g sodium metabisulfite (SO_2)**
  - **iii) 125 mL water and 0.58 mL lactic acid (L.A.)**
  - **iv) 125 mL water, 0.27 g SO_2 and 0.58 mL L.A.**
- **Liquid was separated and analyzed for anthocyanins**
- **HPLC analysis and mass spectrometry for anthocyanin profile and identification**
- **Untreated and room dried pericarp were analyzed**

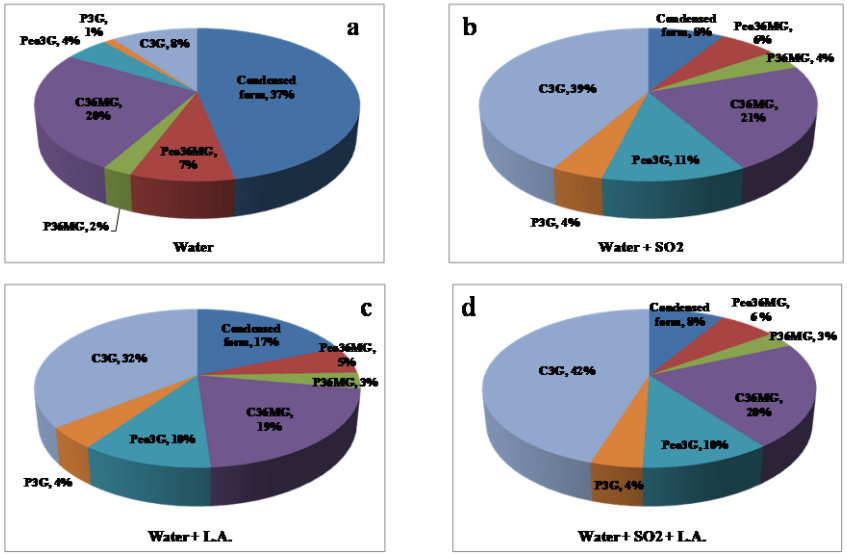


Anthocyanin concentrations



Mean from three replicates; Significance ($p < 0.05$)

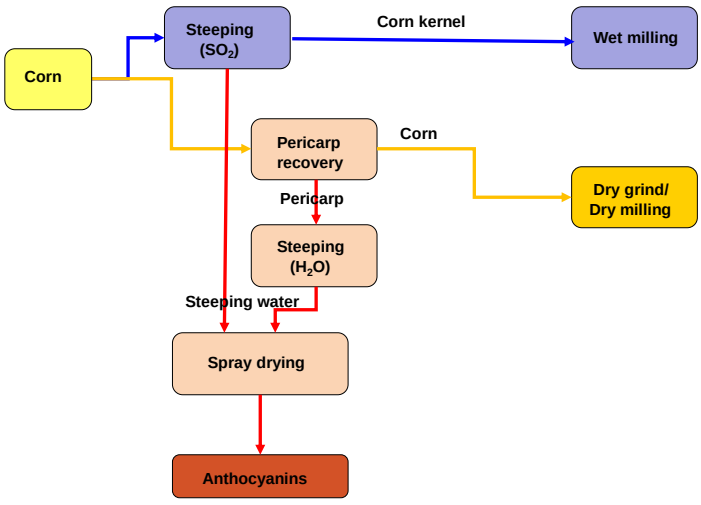
ACN distribution in treatments



Conclusions

- Greater anthocyanins were quantified in treatments with SO_2 (22.9 and 20.5 mg/g pericarp)
- Addition of SO_2 resulted in bleaching of extract color
- Addition of L.A. had no affect on ACN extraction
- Condensed form was dominant in water while in all other treatments, cyanidin-3-glucoside was high
- Total 23 g anthocyanins/kg pericarp were quantified for purple corn making it one of the richest sources (w/w)

Optimal process flow



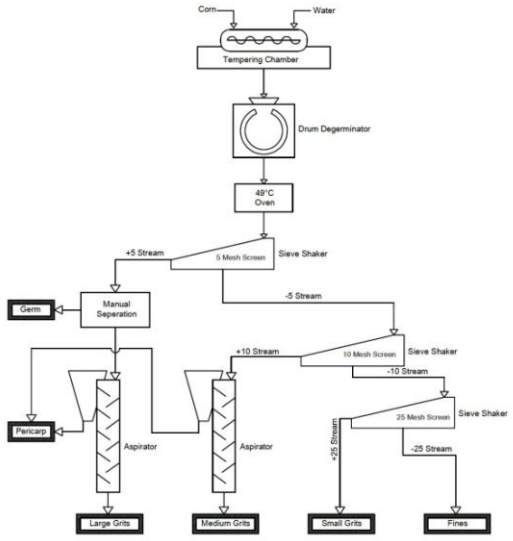
Objective 4

Development of a 100 g dry milling protocol for the measurement of coproduct yield and composition

100 g dry milling protocol

- **There was a need for dry milling samples at 100 g scale; recovery of true sized large grits**
- **A 3x3 factorial design experiment with 20, 30 and 40 min tempering times and 30 min, 1 and 2 hr post-temper drying times was conducted**
- **Twelve experiments with optimized protocol for validating protocol repeatability**
- **Six yellow dent, three colored and a white corn variety were dry milled**
- **Compositional characteristics of corn coproducts were ascertained and compared with commercial samples**

Process flow



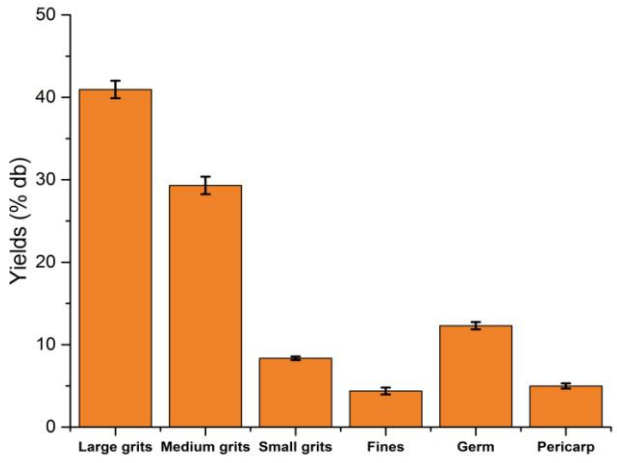
Optimization experiments

	Tempering (20 min)			Tempering (30 min)			Tempering (40 min)		
Drying	30 m	1 hr	2 hr	30 m	1 hr	2 hr	30 m	1 hr	2 hr
LG	42.61a	37.25c	38.3bc	37.88bc	38.38bc	38.22bc	41.75a	37.97bc	40.47ab
MG	27.91b	32.12a	31.82a	28.35b	31.56a	31.91a	27.92b	31.02a	29.91ab
SG	8.47ab	8.85ab	9.00a	8.61ab	8.82ab	8.31ab	8.11b	8.76ab	8.24ab
Fines	4.30a	4.89a	4.84a	4.55a	4.27a	4.48a	4.61a	4.67a	4.63a
Germ	12.09ab	12.10ab	12.05ab	10.26b	12.44a	11.95ab	12.65a	12.42a	12.86a
Pericarp	4.47a	4.73a	4.69a	4.93a	5.00a	5.01a	5.10a	5.02a	5.04a
Total	100.12a	99.94a	100.79a	94.60b	100.50a	99.91a	100.16a	99.88a	101.18a

Mean from three replicates; Means followed by the same letter in a row are not different ($p < 0.05$)

Coproducts

Repeatability study



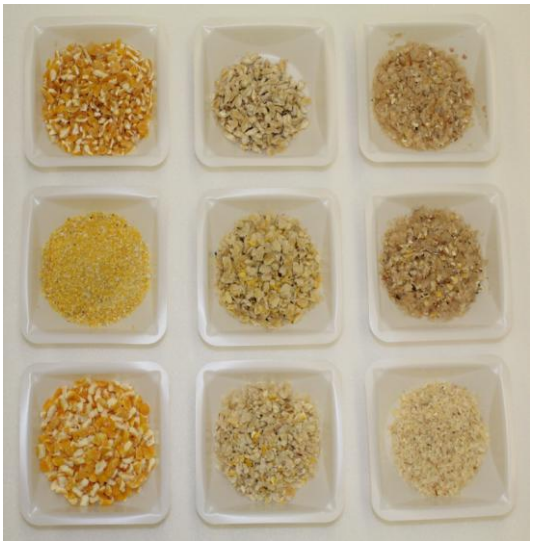
Mean ± SD from twelve replicates

Hybrid characteristics

	LG	MG	SG	Fines	Germ	Pericarp	Total
Hard1	41.12c	30.17e	8.26e	4.21d	12.33ab	4.06f	100.17ab
Hard2	44.21b	23.70g	8.34e	5.69c	12.59a	5.87e	100.39ab
Hard3	46.97a	24.97f	7.75ef	4.19d	11.24bc	5.21e	100.33ab
HE	25.23e	34.85ab	12.67c	8.16a	11.96ab	7.88d	100.75ab
HA	26.38e	33.51bc	11.76d	5.61c	12.94a	11.01a	101.12a
Waxy	31.81d	31.29de	11.69d	6.83b	9.89d	9.10c	100.60ab
Purple	21.34f	34.89ab	14.73b	7.17b	9.68d	13.21a	101.01a
Blue	31.88d	32.70cd	11.79d	3.92d	10.28cd	9.55c	100.12ab
Red	20.87f	35.77a	16.41a	8.16a	9.60d	8.96c	99.78b
White	48.59a	22.67g	7.60ef	5.78c	7.72e	7.82d	100.19ab

Mean from three replicates; Means followed by the same letter in a column are not different ($p < 0.05$)

Coproduct comparison



100 g protocol

1 kg protocol

Commercial

Large grits Germ Pericarp

Conclusions

- A 100 g dry milling protocol for estimating coproduct yield and composition was developed
- Coproduct yields for various hybrids were estimated with low SDs with respect to the means
- True sized large grits were recovered
- Crude oil content of large and medium grits from hard endosperm varieties varied from 0.55 to 1.29% (db)
- Hybrid effects were responsible for variations in coproduct yields and compositions

Overall conclusions

- Suitability of colored cultivars for conventional corn processing industry was evaluated
- Purple corn was found to be the most suitable candidate for ACN recovery
- Effects of SO_2 and L.A. treatment on purple corn pericarp for ACN recovery were studied
- Optimal corn processing methodology for ACN recovery, entailing processing benefits was identified
- A 100 g dry milling protocol for estimating coproduct yield and composition was developed
- Mean coproduct yields were estimated with low standard deviations and true sized large grits were recovered

Future directions

- Milling characteristics and potential of more colored corn varieties need to be ascertained
- Optimization of dry milling parameters for enhancing pericarp recovery from purple corn
- Optimization of pericarp steeping parameters such as temperature, SO_2 quantity and steeping time
- Techno-economic feasibility analysis of incorporating colored corn in conventional corn processing industry

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