TITLE

QUANTIFICATION OF THE POLYPHENOLIC CONSTITUENTS AND RESIDUAL SUGAR OF VARIOUS BRANDS OF BEER

A DESERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE AWARD OF DEGREE OF MASTER OF SCIENCE (MSc.) DEGREE IN INDUSTRIAL BIOCHEMISTRY AND BIOTECHNOLOGY, DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA.

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CERTIFICATION

Ede, Onyemaechi Eze, a postgraduate student with Registration Number PG/M.Sc/17/01727 in the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, has satisfactorily completed the requirements for the course work and research for the award of Masters of Science (M.Sc.) in Biochemistry (Industrial Biochemistry). The work embodied in this dissertation is original and has not been submitted in part or full for any other diploma or degree of this or any other university.

…….. ……

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…….

EXTERNAL EXAMINER

DEDICATION

This work is dedicated to my mother, Mrs. Ede, Theresa

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I appreciate my supervisor Prof. B. C. Nwanguma, an efficient and productive man. You always respond to my challenges and direct me when I am confused. Above all you taught me the principle of confidence. Prof. may God guide you right in all you do.

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ABSTRACT

The study was designed to quantify the polyphenolic constituents and residual sugar of commercial samples of various brands of beer. Fourteen (14) beer samples were randomly selected from different vendor outlets. The total phenolic content of the beer samples analyzed ranged from 35.59 – 87.91 mg/l Gallic Acid Equivalents (GAE) (Mean = 71.69 ± 10.99 mg/l GAE). The highest phenolic content was recorded in sample 7, a lager beer, whose value was, 87.91 mg/l GAE, while sample 13 had the least concentration of 35.59 mg/l GAE. The total tannin concentration ranged from 1.17 – 2.02 mg/ml Tannic Acid Equivalents (Mean = 1.56 ± 0.26 mg/ml), with sample 6 recording the highest concentration, while sample 10 contained the least concentration among the analyzed samples. The total anthocyanin concentration of the samples analyzed was in the range of 3.51 – 6.38 % (Mean = 4.81 ± 0.67 %), with sample 8 containing the highest concentration while the lowest value was recorded in sample 14. Leucoanthocyanin was quantified in each of the samples, and the concentration ranged from 3.5 – 5.1 % (Mean = 4.35 ± 0.41 %). Residual sugar in the beer samples were quantified, and it was found out that the concentration ranged from 2.31 – 6.43 mM (Glucose Equivalents) (Mean = 6.03 ± 1.19 mM). Sample 5 recorded the highest sugar concentration, and sample 14 had the least amount. All the brands analyzed indicated acidic pH, ranging from 4.1 – 5.2, with sample 13 being the most acidic with a pH of 4.1. The differences in the levels of polyphenols, especially tannins account for why some beers are bitter to taste than others. The result of this present study indicates that beer samples contains varying amounts of the phytochemicals: total phenolics, tannins, anthocyanin, proanthocyanin and residual sugar, and that all beer are acidic. This phytochemical assessment could provide information to the consumers about the quality and health benefits of the purchased beer.

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CHD	Coronary Heart Diseases	1
CVD	Cardiovascular Disesase	1
DMACA	Dimethylaminocinnamaldehyde	2
SPE	Solid Phase Extraction	2
HPLC	High Performance Liquid Chromatography	2
LTPs	Lipid Transfer Proteins	11
FFAs	Free Fatty Acids	13
LOX	Lipooxygenase	13
AOS	Alleneoxide Synthase	13
MP1	Metalloproteinase 1	29
COX1	Cyclooxygenase 1	29
BBB	Blood Brain Barrier	32
EGCG	Epigallocatechin gallate	33

CHAPTER ONE

INTRODUCTION

Because of its unique sensory attributes and high nutritional value, beer is one of the most widely consumed beverages worldwide. It is a diverse blend of over 450 constituents including proteins, carbohydrates, polyphenols, fatty acids, nucleic acids and amino acids (Steiner *et al.,* 2011). People have since anciently attributed a variety of health benefits to moderate beer consumption (Ronksley *et al.,* 2011). Many epidemiological studies have also indicated that moderate alcohol consumption decreases overall mortality, predominantly from coronary diseases (Lindberg and Amsterdam, 2008), which may explain the decrease in cardiovascular disease risk (CVD); overall mortality and other diseases found in moderate drinkers. By contrast, alcohol abuse was undoubtedly linked to a large number of medical, social and work-related issues (negative effects), including the development of alcohol-dependence syndrome, several chronic diseases (liver cirrhosis, cardiomyopathy, encephalopathy, polyneuropathy, dementia) and eventually death-related accidents (Estruch and Lamuela- Raventos, 2010; Brien *et al.,* 2011). Several cohort studies have shown that light to moderate drinkers have an increased survival compared to abstainers (Di Castelnuovo *et al.,* 2006). Current evidence also suggests that moderate drinking has protective effects on cardiovascular events including coronary heart disease (CHD), ischemic stroke (Mukamal *et al.,* 2005), peripheral arteriopathy and congestive heart failure. Beer is one of the world's most consumed alcoholic beverages, and is rich in nutrients such as carbohydrates, amino acids, minerals, vitamins, and other compounds such as polyphenols. Hop (*Humuluslupulus*) is one of beer's raw materials that serve as an important source of phenolic compounds. Drained hop cones yield approximately 14.4% of polyphenols, mainly phenolic acids, prenylated chalcones, flavonoids, catechins, and pro-anthocyanidins. About 30 per cent of beer polyphenols come from hops, and 70 per cent -80 per cent come from malt. In addition, hops provide a resin containing monoacyl phloroglucinols that become bitter acids during the process of beer production, such as α-acids (humulones) and iso-α-acids. Simple phenols, benzoic acid derivatives, and cinnamic acid, coumarins, catechins, di- and tri-oligomeric proanthocyanidins, prenylated chalcones and hop-derived α- and iso-α-acids are among the structural groups of polyphenols in alcohol.

Beer was consumed in the middle ages of ancient times for more than 6000 years, from the time it was first made by happenstance. In many cultures it has become a staple part of the diet

(Bamforth, 2002). In addition, it not only included a valuable addition to the table but also served different medicinal roles. Clinical and empirical evidence and laboratory studies have shown that active ingredients in beer can affect the immune system, block cancer formation, protect against coronary disease and even prolong life (Gerhauser *et al.,* 2002).Recent results suggest that beer polyphenols could have beneficial effects on health. Phenolic compounds from beer show good antioxidant activity, especially versus highly reactive hydroxyl radicals in lipid peroxidation processes. In addition, beer can play an active part in preventing Alzheimer's disease and other associated disorders (Gonzalez-Munoz *et al.,* 2008).

The phenolic compounds are naturally present in plants as secondary metabolites. They are of great importance for plant-derived food and drink products, since these compounds are responsible for their organoleptic properties. As a result, they are closely linked to the quality of such products, making their analysis considerably interesting (Goupy *et al*., 1999; Robbins, 2003). Moreover, three groups of polyphenols are responsible forbeer flavour and physical stability (Goupy *et al*., 1999). Simple polyphenols derived from hydroxybenzoicacids (gallic acid, protocatechuic acid, etc.) and hydroxycinnamic acids (ferulic acid, *p*-coumaricacid, caffeic acid, etc.) are extracted mostly from malt but are also present in small amounts in hops. Flavonols (quercetin, kaempferol etc.) come mostlyfrom hop. Flavan-3-ols, including monomers suchas (+)-catechin and (–)-epicatechin,dimers (prodelphinidin B3 and procyanidin B3), trimers (procyanidin C2), flavonoid-derived tanninsup to higher molecular weights, arise equallyfrom malt and hop. The final content of phenolic components of beer depends on both the raw materials and the brewing process.

In quality control, fast analytical methods need to test phenolic compounds, because they can influence the taste and consistency of the beer. Analytical methods in determining phenolic compounds are limited in wort and beer. Several authors determined phenolic compounds in the beer matrices after filtration by direct injection HPLC. Another method is separation of the HPLC and online diode array spectroscopy detection following a chemical reaction with p-dimethylaminocinnamaldehyde (DMACA) (Pascual-Teresa *et al*., 2000). Solid-phase extraction (SPE) is now the common technique used before HPLC separation of phenolic compounds in wines for pre-concentration and purification. (Betes-Saura *et al*., 1996; Guillen *et al*., 2017; Karagiannis *et al*., 2000). HPLC with mass spectrometric detection defined by Robbins (2003), is the most common method of determining polyphenols in wines, olive oils, and other foods and drinks. Reversed liquid chromatography was commonly used for the separation of phenolic

compounds in beer, followed by ultraviolet detection, photodiode-array detection (Sanchez *et al*.,1988; Es-Safi *et al*. 1999), fluorimetric detection (Dvořakova and Dostalek,2006), electrochemical detection (Skerikova *et al*., 2004) or mass spectrometric detection.

1.1 Definition of Beer

Beer is an alcoholic beverage obtained through grain fermentation. Beer is the most consumed alcoholic fermented beverage in the world, in addition to wine. Its history extends from ancient brewers over the German Purity Law from 1516 to modern industrial brewing (Williams, 1996; Wang *et al.*, 2016). Yet beer is characterized not only by its long tradition or its ingredients: (barley) malt, hops, water and yeast but also by its unique characteristics of consistency. The quality characteristic that the customer first identifies is the external appearance of a beer: a shiny, clear sparkling drink with a strong head of foam. The beer's second but most important impression is its flavour. Because of the high export rates in the brewing industry and the consequent longer transport and storage times, the stability of the beer's flavor, color and clarity has become increasingly important. Phenolic compounds contribute to the astringency and colouration of alcohol. Their deciding function in the development of turbidity and their capacity as antioxidants have ensured a continued interest in polyphenol research in brewing science. Beer production involves several processes which ensure that the raw barley is transformed into the beverage.

1.1.1 History of Brewing

Brewing has taken place since around the 6th millennium BC, and archaeological evidence suggests emerging civilizations including Egypt and Mesopotamia brewed beer. Descriptions of various beer recipes can be found in cuneiform (the oldest known writing) from ancient Mesopotamia. In Mesopotamia, the craft of the brewer was the only profession deriving social sanction and divine protection from female deities / goddesses, specifically: Ninkasi, which covered beer production, Siris, who was used metonymically to refer to beer, and Siduri, which covered beer enjoyment. In pre-industrial times, women are often the principal brewers in developing countries (Louis and Oppenheim, 1950).

Since almost any cereal containing certain sugars can undergo spontaneous fermentation due to wild yeasts in the air, it is possible that beer-like beverages were developed independently around the world shortly after a tribal or cultural domestication of cereals. Because nearly any cereal containing some sugars can undergo spontaneous chemical testing of ancient pottery jars

shows that beer was made in what is today Iran as far back as around 7,000 years ago. This discovery shows one of the earliest known fermentation applications and is the earliest evidence to date for brewing. The oldest evidence of beer in Mesopotamia is believed to be a 6,000-year-old Sumerian tablet depicting people drinking a beverage from a communal bowl through reed straws. A 3900-year-old Sumerian poem honoring Ninkasi, the brewing patron goddess, contains the oldest surviving beer recipe, describing barley beer production through bread. The invention of beer and breads have been argued to be responsible for humanity's ability to develop technology and build civilization (Steve, 2007). The oldest chemical-confirmed barley beer to date has been found at God in Tepe in Iran's central Zagros Mountains, where parts of a bottle, at least 5,000 years old, were found to be filled with beer stone, a by-product of the brewing process. Throughout Neolithic Europe, beer may have been identified as far back as 5,000 years ago, and was primarily brewed on a domestic scale.

1.1.2 Classification of Beer

Beer is classified broadly into two based on the type of yeast employed during fermentation. The grouping of beer into different varieties is ancient. Each class of beer is defined by the temperature of fermentation and the strain of yeast used (Dequin, 2001). The two major classes of beer are:

1.1.2.1 Ale

Ales are brewed using top-fermenting strains of yeast (mainly *Saccharomyces cerevisiae*) at temperatures 15 -24 ^{0}C (60 – 75 ^{0}F) which results to full-bodied and fruity tasting beer. The yeast can produce significant amounts of esters and other secondary flavor and aroma products, and the result is often a beer with slightly fruity compounds resembling those found in fruits. In history, ales are believed to be brewed without hops. The bittering agent initially used was gruit, a mixture of herbs or spices boiled in the wort before fermentation (Oladokun *et al.,* 2016). There exist varieties of ale, comprising, brown ale, pale ale, stout, golden ale, Belgian ale, cask ale, mild ale among others.

1.1.2.2 Lager

This beer style is conditioned at low temperatures, 0 – 2 ^{0}C (32 – 36 ^{0}F), using bottom fermenting strains of yeast (e.g *Saccharomyces pastorianus*). Lager beer uses cool fermentation process, followed by maturation in cold storage. Lager is a popular style of beer, and the most common brew in the world and differs from ales because of its brewing techniques that result in

a crisp, refreshing beer profile. Because cold fermentation goes a lot slower than warm fermentation, it takes much longer to make a lager beer (Louis and Oppenheim, 1950). Yeast strains for lager beer drops to the bottom and does the fermentation while ale yeast stays on top of the beer during fermentation. Lager beers are therefore called bottom fermenters and ales top fermenters. Because the lager yeast works at low temperatures, processing the sugars into alcohol takes place at a slow pace (Gupta *et al.*, 2010), leaving very little or no traces of the yeast in the beer.

1.1.3 Stages of beer production

1.1.3.1 Malting and mashing

Malting requires disrupted germination of grains of barley under controlled conditions of humidity, temperature, and time to achieve optimum enzyme yield and structural changes of endosperm in preparation for brewing or food production (Gupta *et al*., 2010). Malting consists of three stages: steeping, kilning and germination. Steeping requires soaking the grains to increase moisture content so that germination can be induced. Germination involves enzyme-mediated endosperm degradation, often referred to as endosperm modification, in which cell walls are depolymerized and proteins stored are catabolized to release starch granules from the matrix. Embryo growth during germination is kept to a minimum in order to reduce the loss of biomass by respiration and by eliminating acrospires and rootlets from the end product. The final stage, kilning, uses heat treatment to stop further germination and conserve enzymes, as well as to create aroma, flavor and colored compounds that help to define the final character of the beer. The task of malting is to produce enough enzymes during malting and mashing to extract a fermentable wort for the hydrolysis of the nutrient reserves in the barley grains (Davies, 2016).

Traditional brewing with barley malt starts when the malt is crushed using roller or hammer mills to produce particles which are more readily removed. The milled product or grist is then mixed with water and heated during the mashing process, which enables the gelatinisation of starch granules and makes them more available to malt enzymes. There are two approaches used for industrial mashing: infusion mashing where the whole mash is heated together, and decoction mashing where part of the mash is separated and boiled before being added back to the rest of the mash to achieve the desired temperature rise (Poyri *et al.*, 2002).Then the liquid (word) is drained from the spent grains using sparkling water, boiling the collected sweet liquid, usually with hops to remove bitterness and flavour. Wort is the resulting sweet liquid at the end of the

mashing which contains fermentable extracts from the digestion of starch, proteins, and other materials. Wort is filtered out of the insoluble spent grains using lauter tuns and grain husks as filter

1.1.3.2 Boiling of Wort

The wort boiling step, which is needed to sterilize wort; precipitate protein complexes; drive off unwanted volatiles; concentrate wort; and, historically, extract bitterness from hops. Many have tried to improve the energy reduction efficiency of the boil without losing sight of its critical importance for the reasons just mentioned, all of which depend on turbulence (vigor) in the boil. A range of novel boiling systems have been proposed, some of which replace thermal energy with gas flow to purge off unwanted flavors (Bamforth, 2009).The insoluble complex generated in boiling (hot break or trub) is removed usually using awhirlpool prior to cooling the wort in a so-called paraflow heat exchanger, in which counter current flow of hot wort and coolant (cold water, propylene glycol) separated by thin metal plates ensures the rapid transfer of heat. Spontaneously fermented beers, such as lambic, are cooled in a shallow vessel open to the elements known as a coolship.

Hops remain the main spice for beer, although an ever-widening range of other materials is apparently being used to add novelty to beer choice. Just as for barley, breeding of new hop varieties is ongoing, with aroma contributions being a particular goal, as well as increased resin levels in those varieties primarily intended to provide bitterness. Hops are quite an intensive crop to grow, not least for their tendency to spoil. There is a particular interest in low-trellis hops, which are easier to cultivate and harvest. As for cereals used in brewing, there is equal interest in the possibilities of organically grown hops (Turner *et al.,* 2011). Some brewers insist that it is only the whole hop cone that provides character to the beer, preferring hops that have not even been dried (which is essential for longer-term storage of the crop). But it is normal for hops to receive some degree of processing to render them easier to handle, and in particular to enhance the recovery of bitterness, which is lost throughout the process, especially from native cones. Most brewers use pelletized hops of various types, though some use hops extracted with liquid carbon dioxide. These can be sub-fractionated into resin and aroma extracts, allowing flexibility in the introduction of bitterness separate from aroma. The resin fraction can be isomerized outside the brewery, adding bitterness to the finished product; still further, such isomerized extracts can be reduced by using hydrogen over a palladium catalyst to produce bitterness

molecules that are no longer converted by light to 3-methyl-2-butene-1-thiol, which delivers a skunky aroma to beer exposed to sunlight. This is why most brewers prefer to use brown glass bottles, though marketing pressure to use green or clear glass packages means that some (though not all) brewers use these reduced side-chain preparations (Oladokun *et al.,* 2016).

1.1.3.3 Fermentation

The filtered wort is processed into beer by fermentation or transformed into malt extract (concentrated wort) for the manufacture of non-alcoholic beverages and other food products. The wort is pitched (inoculated) with yeast after cooling and aeration, and fermented for several days. Yeast cells assimilate fermentable sugars, nitrogen compounds, minerals, and other growth nutrients, and form compounds in the form of esters such as ethanol and higher alcohols, carbon dioxide, and a variety of beer flavours. Maturation takes place after primary fermentation, where the temperature is lowered and the diacetyl off-flavour is reabsorbed and assimilated by the yeast. Progressive development of flavor, yeast sedimentation, and clarification by further precipitation of proteinaceous material (cold break) also occurs during maturation before final filtration and packaging of the beer. Controlled fermentation is the focal point for achieving beer consistency, depending on the production of a consistent yeast growth medium (e.g wort), as well as the employment of the necessary yeast in the correct amounts and in the right condition. Thus, the major fermentation variables are yeast quality and quantity; wort composition and strength, along with oxygen and often zinc additions; temperature; and vessel geometry (Boulton, 2012). Yeast strains differ in their requirement for oxygen for the synthesis of unsaturated fatty acids and sterols that are significant components of the membranes of the cell. Sufficient oxygen must be provided to allow the yeast to multiply to allow the desired fermentation profile and avoid so-called stuck fermentations. However, excessive oxygen leads to overgrowth of yeast, with a shift in the balance or alcohol-to-yeast yield, which is significant in large-scale production (Bamforth *et al.,* 2009).

After 5–10 successive fermentations several brewers should turn to freshly propagated yeast. Whether fresh or not, the yeast pitched into a new fermentation must be viable; this is regularly tested by staining with a dye (methylene blue), while new approaches to determine yeast viability have been of great interest. There is also interest in evaluating the so-called vitality of yeast, namely the health and vigor of living yeast, although none of the methods proposed are still routinely used. Traditional approaches to measuring the concentration of yeasts, particularly

the hemocytometer, are often replaced by instrumental approaches these days. Perhaps the best known are the yeast capacitance measuring devices that are now available for inline use in combination with other measuring probes used in the brewery, such as those used in turbidity, pH, oxygen, carbon dioxide, ethanol and precise gravity checking (Boulton, 2012).

The physical behavior of the yeast is a critical consideration in fermentation. The flocculation phenomenon, in which yeast cells agglomerate to produce flocks which either rise or fall in the fermenter, is highly significant (Verstrepen *et al.,* 2003). Yeast must not separate out from suspension prematurely; if this occurs, fermentation will halt. Similarly, once fermentation is complete, the yeast will flocculate, as this makes clarification processes easier to follow. Flocculation is a characteristic of yeast strain but can also be influenced by composition of the wort, especially calcium levels. Some malts may also provide a catalyst for premature yeast flocculation to wort (Vidgren and Londesborough, 2011).

1.1.3.4 Yeast Strains in Beer Production

One of the world's oldest biochemical processes is the preparation of bread and beer by fermentation using yeasts. Fermentation as a step in the production of beer has a major impact on the enhancement of the final product's shelf life, taste and flavor. *Saccharomyces cerevisiae* (ale yeast) and *Saccharomyces pastorianus* (lager yeast) remain the two principal brewing yeasts. The latter has undergone several name changes and is the more complicated organism that appears to have arisen through a melding of S in the relatively recent past. There are considerably more ale strains than lager strains, especially since the former can be isolated from natural sources. There is a lot of interest in the selection of yeasts which will give beers different character, although the essence of the flavor-active substances produced is similar for most strains, albeit in different proportions. Relatively few brewing strains develop distinct and flavourful entities; may be the best examples are S-strains. Cerevisiae used in the manufacture of hefeweizen products that produce very high levels of isoamyl acetate and relatively unusual 4-vinylguiaicol that has the clove or medicinal character associated with this style of beer (Coghe *et al.*, 2004). This substance is formed by the decarboxylation of ferulic acid by an enzyme coded for by a gene in this form of ale yeast not found in other ale yeasts or lager yeasts, although it may be present in some so-called wild yeasts. The search for flavor diversity has led brewers to explore alternative organisms (Bokulich and Bamforth, 2013). There is nothing new in this; for example, Brettanomyces was a yeast named in honor of the British, who used it to

mature many of their beers, generally referred to as the barnyard. This organism and very many more yeasts and bacteria are spontaneously involved in the production of wild beers, with the best known being Belgium's lambic and gueuze products. The search for diversity or intended process goals has also led to the pursuit of *Saccharomyces* mutants deficient in certain undesirable traits, such as increased sulfur dioxide production capability (Chen *et al.,* 2012) and reduced dimethyl sulfide production capability. (Hansen *et al.*, 2002). Like barley, the ambitions for genetically modified brewing yeasts were thwarted by the inherent reluctance of brewers to use such constructions, even though they were successfully produced and even approved for use.

1.1.3.5 Enzymes implicated in Beer production

Barley undergoes an incomplete germination cycle during malting, which releases or induces enzymes to degrade the endosperm cell wall and releases starch granules from the embedded endosperm matrix. They include α-amylase, β-amylase, limit dextrinases, β-glucanase, xylanase, endo-and exo-proteinases, and lipases (Bamforth, 2009). The hydrolysis of starch into fermentable sugars involves α-and β-amylases and limit dextrinases, and together contributes to the diastatic power of barley malt. α-Amylase is rarely restrictive unless brewing with a high proportion of raw grain adjuncts; β-amylase is present in raw barley but requires release from its bound form (inhibitor protein) for optimal access to the substrate; limit dextrinase activity may also be inhibited by the presence of protein inhibitors in malt. Both xylanase and β-glucanase are important components of the endosperm cell wall, and thus are essential for nutrient mobilization. Proteases not only increase the free content of amino nitrogen (FAN), but also enhance the production of fermentable sugars by destroying limit dextrinase and β-amylase inhibitor proteins. When brewing beer with 100% malted barley, the endogenous enzyme levels are usually high enough to produce good quality wort; however, the use of raw barley in brewing is beneficial in terms of cost, CO_2 emission, and the availability of quality barley for the brewing industry.

1.1.3.6 Brewhouse operations

Malt has to be stored several weeks before use; otherwise, brewery solid-liquid separation operations are in jeopardy. It has been proposed that there is a decrease in the level of enzyme (s) in malt during this storage that encourages the bridging of high-molecular weight molecules that form a system that impedes the flow of liquids (Bamforth *et al.,* 2009). In addition to brewers operating on a very large scale, most brewers grind the malt using two rolling mills, sometimes with prior steam treatment of the malt to make the husk more flexible or even to the extent of so-

called wet friction, where the malt is soaked several minutes before grinding. The goal of any roller milling operation is to keep the husk as intact as possible, which forms a filter bed in the wort separation system, while ensuring that the starch endosperm is crumbled into flour and finer grits to optimize the possibilities for hydration and the extraction of enzymes and substrates.

However, there is growing interest in wort-separation systems which are not dependent on the husk, particularly the mash filter. When such a device is installed, it is necessary to pulverize the malt as much as possible to maximize material extraction which is accomplished with a hammer mill. The increased extraction of materials from very small particles naturally includes those which are unwanted. Modern brewhouses are designed to minimize the influx of air, because oxygen is of concern as a source of enzyme browning, and can reduce the consistency of the beer's flavor. (Vanderhaegen *et al.,* 2006). Nevertheless, reductions in air intake are usually accomplished by using intimate mixing stems; low entry into vessels; and deaeration of milled grist and/or brewing water for some. The invention of mash mixers with steam jackets and rousers (agitators) made historical approaches to adjusting the temperature of mashes unnecessary — with low temperature to complete the degradation of cell wall materials and protein (although there are those who contend that proteolysis is minimal in mashing, accompanied by a higher temperature that is adequate to gelatinize starch while allowing enzyme survival. Use of very well modified malt means it should indeed not be strictly necessary to have a low temperature commencement to mashing (Coghe *et al.,* 2004). One strategy to avoid a low-temperature mash, even for less-well-modified grists, is to employ heat-tolerant enzymes of microbial origin. But there are those who resist this, as they believe that enzymes endogenous to malt are preferable.

While traditional mash tunes, in which mashing and wort selection are carried out in one vessel, remain in use in some breweries, most brewers keep these stages separate, with a mash mixer accompanied by a lauter tun or mash filter. In both cases, wort selection takes place via Darcy's rule, with particular emphasis on large surface area (wide lauter tunes, several mash filter plates) and low bed depth (shallow grain beds in lauter tunes, narrow chambers in mash filters); Excessive pressure in a lauter tuning can lead to blockage of the holes in the platers, whereas pressure is essentially an advantage in the mash filter, allowing squeezing out of wort and therefore shorter turnarounds. Comparison was made of the relative benefits and disadvantages of these two schemes. In both cases the coproduct is spent on grains whose high content of

moisture demands that they be shipped as soon as possible. Drying them is inexpensive, and they are most widely used as cattle food, though a number of other possibilities have been proposed (Bamforth, 2009). A radical approach would be to split the grist premashing into a dry spent grain product, although this did not attract commercial attention. However, efforts have been made to minimize husk contribution to features such as increased astringency due to their polyphenol content through selective milling approaches.

1.1.3.7 Water in beer production

Although much more water is used in planting crops (barley, wheat, adjuncts and hops) and in malting than in brewing, brewing is still viewed as a water-intensive process. It is now entirely feasible to have a water-use ratio (liters of water per liter of packaged beer) of 3:1 or even higher in brewery wall operations. Water entering the brewery is usually treated to make it free of taints and to ensure that it possesses the composition deemed suitable for the beers made. (Bokulich and Bamforth, 2013). Water will also be treated to remove hardness before use in steam generation systems and will be deaerated when used for various brewery operations, notably when adjusting the alcohol content in high-gravity brewing. For such operations, hydrophobic membrane-based systems are available, as they are also used to adjust the gas content of finished beer. Wastewater is also increasingly handled on-site by brewers through aerobic and anaerobic digestion systems, the latter offering opportunities for energy recovery. Indeed, diverse systems, including wind and solar power, are generally used in breweries to maximize energy recovery.

1.1.4 Proteins in beer

Beer has 0.5–1g / l molecular weight proteins between 5 and 100 kDa. The main beer proteins are involved in haze formation and foam stability, two essential criteria that determine the consumer's experience of a beer. Recently an overall view of beer proteins was given using the current strategy of proteomic, two-dimensional electrophoresis coupled with mass spectrometry. Most data converge with two main beer proteins: protein Z and lipid transfer proteins (LTPs). Protein Z is a protein with a molecular mass of 45 kDa protein (Jegou *et al.*, 2001). The second major beer protein, LTP, is composed by two proteins, LTP1 (about 9 kDa) and LTP2LTP1 is that family's most abundant protein. Other minor proteins were highlighted as protease and amylase inhibitors. In beer, enolase and triose phosphate isomerase have also been identified as enzymes that play a major role in the metabolism of yeast cells. Except for hordeins, most of the proteins identified are soluble proteins (i.e., albumins) as predicted from malted barley aqueous extraction. Not all soluble barley proteins are recovered in beer, however, during beer maturation

most of these proteins precipitate in part by forming large aggregates of hop and malt compounds. These complexes are responsible for creating haze in beer.

LTPs (i.e. LTP1 and LTP2) are ubiquitous proteins that bind lipids in plants. They form a multigenic family in which the expression of the different proteins is regulated spatially and temporally. This is well illustrated for wheat seeds, where the nine LTPs are expressed at the various stages of the grain production. At the last stage of grain development, the major barley and wheat seed LTP1 and LTP2 are expressed in the aleurone cells (Boutrot *et al.*, 2005). Four disulfide bonds stabilize those helical proteins. There is a broad hydrophobic cavity within the protein or a tunnel that is the binding site of lipids. Such proteins can bind all kinds of lipids and hydrophobic molecules. In general, lipid binding involves non-covalent lipid-protein bonds. Recently, however, it has been shown that barley LTP1 can also bind covalently oxygenated fatty acid derivatives (i.e. polyunsaturated fatty acid oxidation products) also known as oxylipins (Bakan *et al.*, 2006). A specific site has been highlighted for these oxylipins. The biologicalrole of LTPs is still unknown but most data converge for a role of these proteins in the defense signaling of plants against microbial pathogens. Some LTPs are capable to inhibit fungal growth by permeabilizing the membranes of the pathogens (Blein *et al.*, 2002). These proteins may also inhibit protease and endo-proteases of barley in particular. LTPs are also ubiquitous allergens to plant foods and the corresponding beer proteins were described as a major allergen in rare patients. These proteins are highly stable for heat treatments and only a long boiling or heating procedure in the presence of reduction agent allows their denaturation (Van Nierop *et al.*, 2004). As for protein Z these proteins are not glycosylated in barley seeds but are glycated in beer. LTP glycation occurs at the last kilning stage by Maillard reaction when the green malt hydration decreases and kilning temperature increases. Most of the LTP1 is unfolded in beer and some bonds to all disulfide are broken (Jegou *et al.*, 2001). Interestingly, there may be some rearrangement of disulfide bonds, and a chimeric protein associated with LTP1 and LTP2 was observed in beer.

1.1.4.1 Beer proteins and haze formation

Brilliance or clarity (i.e., absence of a haze or sediment) is considered by most consumers to be an important criterion for accepting beer. Haze is usually formed by cold conditioning of freshly fermented beer and can be caused by multiple materials such as yeast cells and organic or mineral colloidal particles. Much research has been done to characterize the haze-forming

materials in beer as well as in other drinks by analyzing the sediment's chemical composition. Significantly, haze-forming molecules consist mainly of polypeptides derived from the storage of proline-rich barley endosperm proteins (i.e.hordeins) and malted barley and hop polyphenols. Another protein, the barley trypsin inhibitor, which is notable for proline-rich hordein protein, also appears to play a role in the formation of beer haze (Robinson *et al*., 2007). Although carbohydrates are present in the sediment in high quantities, it has been shown that they do not participate in haze formation but co-aggregate with haze particles. Haze is primarily due to the development of cold conditioning protein and polyphenol aggregates. The complex of polyphenols – proteins grows to sufficient size to induce turbidity and can eventually form large particles that can sediment (Steiner *et al.*, 2011).

1.1.4.2 Proteins and beer foam

Head foam formation and consistency strongly influence customer perception of beer quality. Proteins are key in forming a visco-elastic film around gas bubbles in a beer's foaming properties (Maeda *et al.*, 2003). Other factors may be affecting beer foam formation and stability. Ethanol for example increases the formation of foam but reduces its stability. Hop acids improve the stability of foam in forming protein complexes at the interface between gas and liquid. Lipids are the primary foam-negative liquid. At the interface, these hydrophobic and/or amphiphilic molecules quickly adsorb and destabilize the protein film by displacing protein and decreasing protein – protein interactions. This leads to foam bubbles breaking down and coalescing.

The method of malting and brewing converts the albumins in the barley into foaming proteins. Recently, the mechanism involved in this structural maturation has been delineated particularly for one of the major beer proteins, LTP1. The barley LTP1 does not actually form any foam whilst the corresponding beer protein shows good foaming properties. LTP1 in beer is a mixture of glycated proteins that show different unfolding state (Jegou *et al*., 2001). During the malting process, glycation occurs on kilning while the brewing is unfolding (Van Nierop *et al.*, 2004). Heat-induced unfolding of LTP1 is strengthened by raising the protein's disulfide bonds during malt extraction. The malt reduction mechanism of the disulfide bond has not yet been identified but it should involve the pathways that reduce thioredoxin and/or glutathione oxide. Another significant modification is the LTP1 acylation. Two embryo enzymes, a9-lipoxygenase (9-LOX) and an alleneoxide synthase (AOS) catalyze this acylation. 9-LOX produces9-hydroperoxide from linoleic acid, the major poly unsaturated free fatty acids (FFAs) from cereal seed, while the

AOS transforms the hydroperoxide into the corresponding allene oxide. The electrophilic attack of unstable allene oxide by the protein's acidic side chain (Asp7) results in a covalent binding of an alpha-ketol to LTP1. This acylation for the glycated LTP1 was observed in beer (Jegou *et al.,* 2001). The barley LTP1 can be covalently bound to up to two alpha-ketols. Finally, glycation and acylation increase the amphiphilic character of the protein while unfolding improves the spreading of the protein at the air-water interface, allowing a better anchoring of the interface through the acyl adducts and the formation of protein-protein interaction in the film surrounding gas bubbles. Lipids, and particularly FFAs, are detrimental to beer foam formation and stability. LTP1 trapping of the main barley FFA (i.e. linoleic acid) by LOX and AOS combined action will reduce the accumulation of foam-destabilizing FFAs. With the non-covalent lipid binding (Cooper *et al.,* 1998), this mechanism may act synergistically to protect beer against lipid-induced foam destabilization. Lastly, it should be stressed that the quality of beer foam is not due to a unique protein but to complex associations between the different beer proteins and between proteins and other components (phenolic compounds, hop acids, minerals, etc.) as well as to technological processes (i.e. malting and brewing) that induce various structural modifications that are essential in order to generate pro-foaming entity (Jegou *et al.,* 2001).

Over-foaming can be produced when packets are opened. This phenomenon called gushing is one of the major sources of the brewers ' economic losses. Primary spraying is caused by factors arising from malt or other cereal raw material that enter the brewing process. Secondary gushing is due to the presence of aggregates and particles related to impurities or haze in the technology. Primary gushing is closely related to malt fusarium contamination and is presumed to be due to active surface proteins that form a highly stable film around gas bubbles. Most studies converge for the role of tiny fungal proteins (i.e. hydrophobins) (Jung *et al.,* 2007). Those fungal proteins are highly stable with four disulfide bonds. They can survive heating on brewing and only small amounts of these proteins can lead to sprinkling. These proteins display an amphiphilic character that allows for rapid adsorption to form stable and ordered aggregated protein films with unique elasticity at the gas – water interface. It could also involve the modified forms of LTPs (i.e. glycated and acylated proteins) in gushing. These proteins share structural features with wort-isolated gushing factor. Though these proteins are essential to beer's foaming properties, they may induce gushing above a concentration threshold. Since most LTPs are synthesized to target pathogenic agents (Blein *et al.,* 2002) can produce higher levels of LTPs in response to barley fungal infection. The role of LTPs in gushing, alone or interacting with fungal hydrophobins has

not yet been highlighted, but needs to be investigated with a view to improving the quality of beer foam by growing LTP content through either barley breeding or genetic modifications.

1.1.5 Inorganic salts in beer

Salts in beer come from brewing water and/or malt and they are effective on the consistency of the beer. Calcium, magnesium, sodium, potassium, sulphate, chloride, bicarbonate, carbonate and nitrate are the principal ions present in most brewing liquors (Johnson *et al.,* 1994).Ironacts as a pro-oxidant and thus increase the stalling of beer, consequently, its presence is discouraged by brewers. Calcium ion (Ca^{2+}) is the most important mineral in the brewing process because it affects the wort's acidity; it can react with malt phosphates and can form primary, secondary or tertiary phosphates. The secondary phosphate is weakly soluble while tertiary phosphate is isoluble. The more calcium present in the wort the more tertiary phosphate is produced that is accompanied by a release of hydrogen ions which makes the wort more acidic (Holopainen, *et al.,* 2014).

$$3Ca^{2+} + 2(HPO_4)^{2-} \longrightarrow 2H^{+} + Ca_3(PO_4)_2$$

The pH reduction is important because it provides an ideal environment for proteolytic enzymes, called α-and β-amylase. This will in turn increase saccharification and proteolysis, resulting in increased extract yield and soluble nitrogen. Reducing the pH can lower the viscosity of the wort enough to faster filtration. In addition, polyphenols (so-called tannins) are processed at a lower pH to a lesser degree, resulting in less astringent beers with less colour. Calcium enhances the yeast flocculation and sedimentation during primary fermentation and increases the clarity, stability and flavor of the final product during maturation (Nakajima *et al.,* 2001). Calcium ions cause malt-derived precipitation of oxalate that tends to precipitate during the brewing process. If these ions are not sufficient, oxalate precipitation may be delayed and may cause haziness and foam problems in the finished beer in some cases. Usually a 4:1 ratio Ca:oxalate is recommended but some brewers advocate 10:1 ratio to prevent haze formation. Too much calcium, however, can lead toan intense precipitation of phosphates causing a wort poor of a vital yeast nutrient.

Carbonate $(CO_3)^{2-}$ and Bicarbonate $(HCO_3)^{1-}$ take up hydrogen ions (H^{+}) from the wort to produce water and carbon dioxide (CO_2). When calcium bicarbonate is present in water producing temporary hardness the detrimental effects ofthe bicarbonate ions outweigh the beneficial effects of the calcium ions. Alkalinity is a measure of the buffering capacity of the bicarbonate ions and, to some extent, the carbonate and hydroxide ions of water. These three ions

all react with hydrogen ions to reduce acidity and raise pH (Liu *et al.*, 2013). Alkalinity is normally given in mg/l (mg/l) as calcium carbonate ($CaCO_3$) for all three ions. Usually water with more than 100 mg/l of calcium carbonate is considered alkaline and should be treated. Water with less than 100 mg/l of calcium carbonate is considered soft or just slightly alkaline (Rizvi and Zaid, 2005). The effect of carbonate ions in raising pH can give less fermentable worts due to a higher dextrin/maltose ratio, slow wort filtration and less efficient separation of protein–polyphenol compounds during the hot and cold break. Moreover, high carbonate concentration can have a negative influence on hop flavor while hop bitterness becomes harsher. Usually carbonate concentration should not exceed50 mg/l to avoid harsh and bitter flavors. Carbonate in excess of 200 mg/l is acceptable only when a dark roasted malt is used to buffer its excessive acidity.

Magnesium (Mg2 +) has similar properties to calcium ions and also reacts in malt with phosphates but the phosphates that are formed are more soluble. Thus less hydrogen ions are emitted and hence less acidity increases. Magnesium ions are important as enzyme cofactor for the yeast metabolism that is required in very small amounts. Malt and water usually contain enough magnesium to provide the necessary quantity. At concentrations above 15 mg / l, they can also add a sour or bitter astringency to the beer. Manganese ($Mn^{2+)}$ functions as a cofactor of enzymes and a positive action for solubilizing proteins. This ion can inhibit the metabolism of yeast and negatively affect the colloidal stability of beer. The amount of manganese should be 0.1 to 0.2 mg / l in wort, and no more than 0.5 mg / l in any case (Margaret *et al.*, 2018).

Sodium (Na+) affects sweetness and smoothness at low concentrations (ranges from 70 to 150 mg /l) which is most nice when combined with chloride ions compared to sulfate ions. Higher concentrations offer a salty and discomforting taste (Rizvi and Zaid, 2005). The combined effect of the ions on the beer when sodium sulfate is present in the brewing liquor is an undesirable harshness and sour taste; the upper limit of this salt for brewing water is known to be 150 mg / l. Many breweries tend to use potassium chloride rather than sodium chloride, since potassium is free of the sodium ion's slightly sour taste. However, when sodium chloride is applied, fullness is created at the appropriate concentration palate. Potassium (K^+) possesses similar properties to sodium ions but does not give sodium ions softness to beer. Thus, potassium chloride is commonly used in addition to sodium chloride if chloride ions need to be added in a water treatment. It is necessary for metabolism of the yeast, and inhibits some mash by more than 10 mg / l.

1.2 Beer raw plant material

Cultivated barley is part of the genus Hordeum vulgare, a monocotyledonous flowering member of the Poaceae family of grasses. Barley grain consists of several protective tissue layers including an outermost husk, pericarp (fruit wall) and testa (seed coat) enclosing an embryo and a large starchy endosperm, with a scutellum that separates the embryo from the endosperm (Holopainen *et al.,* 2014). The endosperm is the most interesting structure, as it contains the nutrient reserves of the grain that will be extracted during fermentation for yeast nutrition and production. The endosperm forms approximately 75% of the weight of the barley grain and consists of an outer aleurone layer of living cells lined with proteins and lipids and an inner cell filled with starch granules embedded in a matrix of storage proteins. As for the chemical composition, starch granules consist almost entirely of polysaccharides of amylose and amylopectin. Amylose consists primarily of α-(1,4)-linked long linear chains of glucose residues, while amylopectin has relatively shorter chains and is extensively branched by α-(1,6)-glucosidic bonds. Matrix storage proteins are the predominant proteins in endosperm and have a high content of proline and glutamine, and are thus collectively known as prolamins in cereal grains or specifically as barley hordeins (Balakireva and Zamyatnin, 2016). Endosperm cell walls consist mainly of aleurone β-glucans and heteroxylan (mainly arabinoxylan) at 26% and 71% respectively, and 70% and 20% respectively (Burton and Fincher, 2014).

Nutrient mobilization during germination takes place through enzymatic endosperm hydrolysis; some of these enzymes are already present in aleurone, but most of the enzymes are de novo synthesized by aleurone during germination. (Palmer and Duffus, 1986). The malting process involves basically interrupting the germination of barley grains under controlled conditions of humidity, temperature and time to achieve optimal enzyme yield and structural changes of the endosperm in preparation for brewing or food production (Gupta *et al*., 2010).

Barley is an ample source of phenolic compounds, and an outstanding dietary matrix of natural antioxidants can be considered for disease prevention and health promotion. In addition, phenolics in barley are very important because of their impact in various stages of the brewing process and the overall stability of the beer (Whittle *et al.,* 1999; Coghe *et al.,* 2004; Iyuke *et al.,* 2008). It is known that over 60% of the total polyphenolic content found in beer comes from barley (Quifer-Rada *et al.,* 2015). Barley contains various groups of phenolic compounds, such as derivatives of benzoic and cinnamic acid, proanthocyanidins, quinines, flavonols, flavanones,

and amino phenolic compounds. We can be found in a free, esterified, or insoluble bound form, and are distributed quantitatively between different grain tissues.

1.3 Polyphenols

Polyphenols are secondary plant metabolites and are typically involved in the defense against ultraviolet radiation or pathogenic aggression. They are used mostly in fruits, vegetables, cereals, and drinks. Fruits such as raisins, apples, pears, cherries and berries contain up to 200–300 mg of polyphenols per 100 g fresh weight. The products produced from those fruits also contain significant amounts of polyphenols. A glass of red wine or a cup of tea or coffee will typically contain around 100 mg of polyphenols. Cereals, chocolate and dry legumes also contribute to the polyphenolic intake (Scalbert *et al.*, 2005; Spencer *et al.*, 2008).

Polyphenols in food can contribute to the stability of bitterness, astringency, colour, taste, odour, and oxidativeness. By the end of the 20th century, epidemiological studies and related meta-analyses strongly suggest that long-term dietary consumption rich in plant polyphenols provided some protection against cancer growth, cardiovascular diseases, diabetes, neurodegenerative diseases and osteoporosis (Graf *et al.*, 2005; Arts and Hollman *et al.*, 2015). Polyphenols and other phenol containing foods are the subject of increasing scientific interest due to their possible beneficial effects on human health.

1.3.1 Occurrence and Content of polyphenols

Distribution of phenolics in plants at the tissue, cellular and sub cellular levels is not uniform. Insoluble phenolics are found in cell walls, while soluble phenolics are present within the plant cell vacuoles (Wink, 1997). Certain polyphenols like quercetin are found in all plant products; fruit, vegetables, cereals, fruit juices, tea, beer, wine, infusions etc., whereas flavanones and isoflavones are specific to particular foods. In most cases, foods contain complex mixtures of polyphenols. The outer layers of plants contain higher levels of phenolics than those located in their inner parts (Simon *et al.*, 1992). Numerous factors affect the polyphenol content of plants and these include degree of ripeness at the time of harvest, environmental factors, processing and storage. Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall etc. The degree of ripeness considerably affects the concentrations and proportions of various polyphenols (Manach *et al.*, 2004). In general, it has been observed that phenolic acid content decreases during ripening, whereas anthocyanin concentrations increase. Many polyphenols, especially phenolic acids, are directly

involved in the response of plants to different typesof stress: they contribute to healing by lignifications of damaged areas possess antimicrobial properties, and their concentrations may increase after infection (Parr and Bolwell, 2000). Another factor that directly affects the polyphenol content of the foods is storage. Studies have proved that polyphenolic content of the foods change on storage, the reason is easy oxidation of these polyphenols (Manach *et al.*, 2004). Oxidation reactions result in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics. Such changes may be beneficial, as is the case with black tea or harmful as in browning of fruit. Storage of wheat flour results in marked loss of phenolic acids (Sosulski *et al.*, 1982). After six months of storage, flour contained the same phenolic acids in qualitative terms, but their concentrations were 70% lower compared with fresh flour. Cold storage, in contrast, has slight effect on the content of polyphenols in apples, pears or onions (Price *et al.*, 1997). Cooking also has a major effect on concentration of polyphenols. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying. (Crozier *et al.*, 1997).

1.3.2 Classification of Polyphenols

More than 8,000 polyphenolic compounds have been identified in various plant species. All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Primarily they occur in conjugated forms, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar (polysaccharide or monosaccharide) to an aromatic carbon also exist. Association with other compounds, like carboxylic and organic acids, amines, lipids and linkage with other phenol is also common (Kondratyuk and Pezzuto, 2004). Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes, lignans etc. (Spencer *et al.*, 2008).

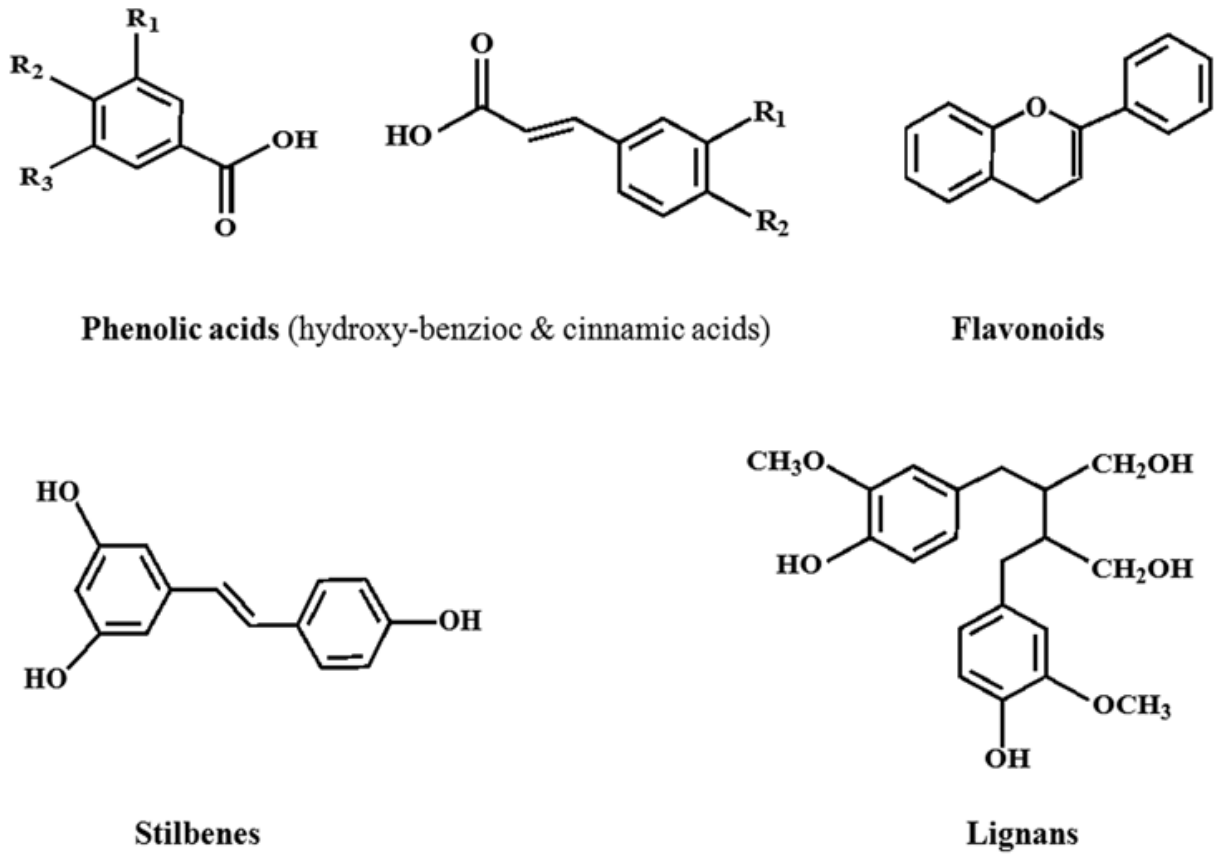


Figure 1. Chemical structures of the various classes of polyphenols (Spencer *et al.,* 2008)

Barley contains an assortment of phytochemicals (non-nutrient components) in varying concentrations usually determined by genotypic or environmental factors, or the interaction of both factors. Phytochemicals in barley may exist in free, conjugated, or bound forms and are categorized into several major classes (Fogarasi *et al.,* 2015).

1.3.2.1Phenolic acids

Phenolic acids, the dominant phenolic group of phytochemicals in barley are primarily located in the outer layers of the kernel (Dykes and Rooney, 2007). They are subdivided into two groups: benzoic acid and cinnamic acid and their derivatives. Phenolic acids have been linked to chronic disease prevention partly due to the presence of unsaturated carboxylic group (Pandey and Rizvi, 2009). The abundant content of phenolic acidsin barley, especially in the hulled variety, indicates that itmay also serve as an excellent dietary source of natural antioxidants with antiradical and antiproliferative potentials (Quinde-Axtell and Baik, 2006). In barley, phenolic acids are found at highest concentrations in the bound form, followed by conjugated and free forms, respectively. The free forms are often located in the outer part of the pericarp, whereas the bound forms are

esterified to cell wall constituents, such as lignin, cellulose, arabinoxylans, polysaccharides, and hemicelluloses. Free phenolic acids are usually a small portion of the total phenolic acid concentration. The phenolic acid concentration in barley approximately ranges between 4.6 mg/g and 23 mg/g for the freeform, between 86 mg/g and 198 mg/g for the conjugated form,and between 133 mg/g and 523 mg/g for the bound form, whereas the total phenolic acid concentration ranges between604 mg/g and 1346 mg/g. The free forms of the major phenolic acids in barley are ferulic acid (FA; 27% of dry matter), vanillic acid (28%), syringic acid (17%), and p-coumaric acid(22%) (Gamel and Abdel-Aal, 2012).

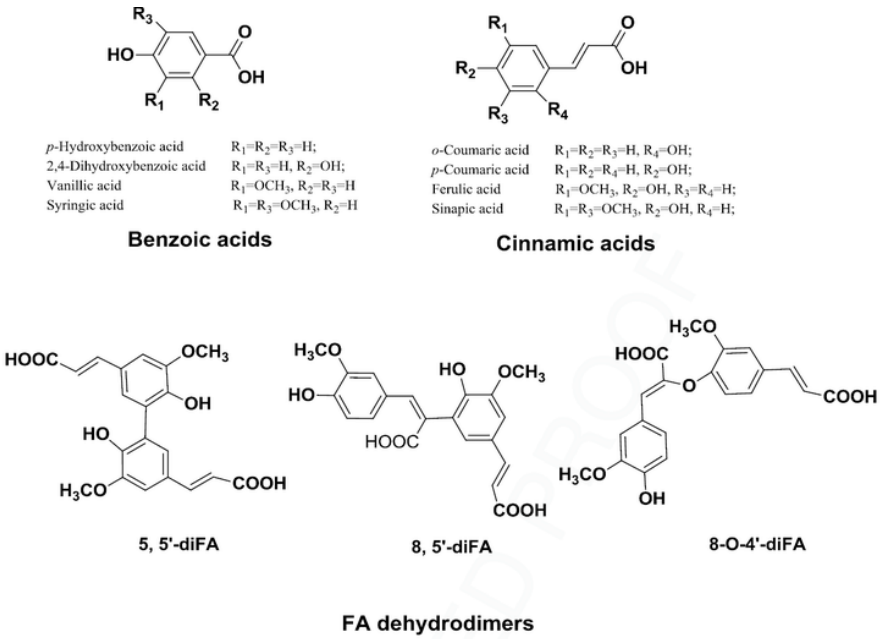


Figure 2. Phenolic acids in barley (Barone *et al.*, 2009).

FA is the most abundant and the main low molecular weight phenolic acid in barley (Andersson *et al*., 2008). It accounts for approximately 68% of total phenolic acids in barley, and its concentration in barley grains ranges between 149 mg/g and 413 mg/g (Ward *et al.*, 2008). Andersson *et al* (2008) reported that the total concentration of FA in barley is about 270 mg/g of dry matter, whereas the average total concentrations of free, conjugated, and bound FA for different varieties of barley were approximately 2.7 mg/g, 33.21 mg/g, and 235 mg/g, respectively (Andersson *et al*., 2008).

The hydroxybenzoic acid content of edible plants is generally low, with the exception of certain red fruits, black radish and onions, which can have concentrations of several tens of milligrams

per kilogram fresh weight. The hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic and sinapic acids.

1.3.2.2 Flavonoids

Flavonoids are phytochemical compounds with a C6-C3-C6 skeleton. They have two aromatic rings bound together by three carbon atoms that forman oxygenated heterocycle. More than 4,000 varieties of flavonoids have been identified, many of which are responsible for the attractive colours of the flowers, fruits and leaves (de Groot and Rauen, 1998). They are also believed to be UV-B absorbing compounds, which provide protection against UV radiation in response to "excess light" stress. Clinical studies indicate that flavonoids may be the bioactive substances present in cereal grains responsible for the moderation of many diseases including cancer and coronary heart diseases. Flavanols, anthocyanins, and proanthocyanidins (polymers of flavonoids) are the major types of flavonoids found in barley grains. Flavanols and anthocyanins are located in the pericarp of barley grains where they exist mostly as glycoside derivatives, including cyanidin-3-glucoside, penidin-3-glucoside, and delphinidin-3-glucoside. Individual differences within each group arise from the variation in number and arrangement of the hydroxyl groups and their extent of alkylation and/orglycosylation (Spencer *et al.,* 2008). Quercetin, myricetin, catechins etc., are some most common flavonoids. Generally, the content of flavonoids in barley grains are proportional to the degree of color depth, and blue and purple barley grains have been discovered to possess the most flavonoid content among barley varieties (Liu *et al.,* 2013).Kim *et al* (2001) studied the flavonoid content of 127 lines of hulled and unhulled colored barley wherein the total flavonoid content was found to range between 62.0 and 300.8 mg/g.

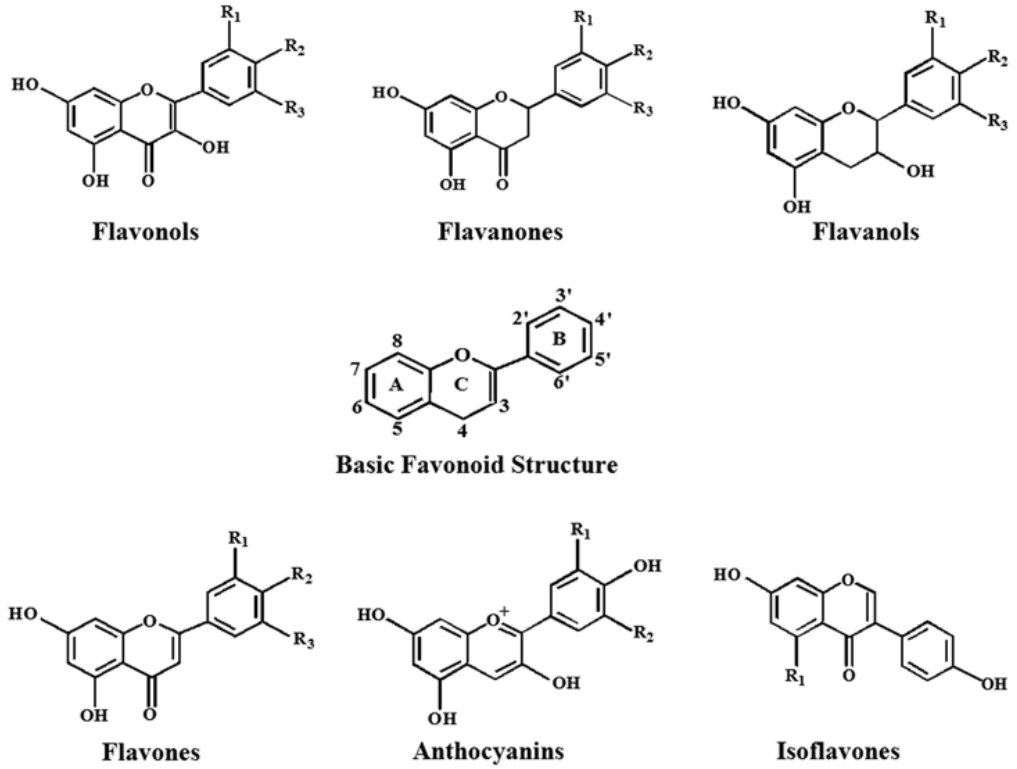


Figure 3. Chemical structures of sub-classes of flavonoids (Kim *et al.*, 2001).

1.3.2.3Lignans

Lignans are diphenolic compounds that contain a 2,3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues. Several lignans, such as secoisolariciresinol, are considered to be phytoestrogens. The richest dietary source is linseed, which contains secoisolariciresinol (up to 3.7 g/kg dryweight) and low quantities of matairesinol. Lignans are natural polyphenols widely distributed in the plant kingdom as natural defense substances. They are bioactive as phyto-estrogens because of their structural and functional similarity to 17b-estradiol. Lignans have been suggested to induce a wide range of biological effects, such as antioxidant, antitumor, antiviral, antibacterial, insecticidal, fungistatic, estrogenic, and antiestrogenic activities, and protect against coronary heart disease (Rhee, 2016; Prasad and Jadhav, 2016). There is little information in the literature about lignans in barley. The content of lignans in barley wasreported to include pinoresinol (71 mg/100g), medioresinol(22 mg/100g), syringaresinol (140 mg/100g), lariciresinol (133 mg/100g), cyclolariciresinol (28 mg/100g), secoisolariciresinol (42 mg/100g), secoisolariciresinol-sesquilignan (24 mg/100g), matairesinol (42 mg/100g), oxomatairesinol (28 mg/100g), and 7-hydroxymatairesinol (541 mg/100g) as

major lignans and todolactol (127 mg/100g), a-conidengrin acid (33 mg/100g), nortrachelogenin(15 mg/100g), and lariciresinol-sesquillgnan (6.6 mg/100g) as minor lignans.

1.3.2.4 Stilbenes

Stilbenes contain two phenyl moietiesconnected by a two-carbon methylene bridge. Occurrence ofstilbenes in the human diet is quitelow. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury (Manach *et al.*, 2004). One of the best studied, naturally occurring polyphenol stilbene is resveratrol (3,4',5-trihydroxystilbene),found largely ingrapes. A product of grapes, red wine also contains significant amount of resveratrol.

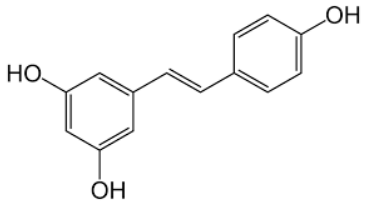


Figure 4. Resveratrol (3,4',5-trihydroxystilbene) (Young *et al.*, 1999)

1.3.2.5 Tocols

Tocopherols and tocotrienols collectively known as tocols; are a class of lipid-soluble phytochemicals found in barley. Tocols are recognized for their antioxidant properties, especially their ability to inhibit lipid peroxidation in biological membranes. In addition to their antioxidant properties, tocols found in cereals proffer anticancer and cancer suppression effects, induce the immune system, moderate the risk factors of cardiovascular diseases(CVD), and promote apoptosis induction (Suman *et al.*, 2013).One of the most striking discoveries about tocols is their ability to clear atherosclerotic blockages (stenosis) in the carotid artery, potentially reducing the risk of stroke (Upadhyay and Misra, 2009).Barley is one of the best sources of tocols among cereals due to a high concentration and favorable distribution of all eight biologically active vitamers. Moreau *et al.,* (2007) reported that levels of total phytosterols and total tocotrienols in the oils prepared from both whole kernels and scarified fines of barley ranged between 2911 mg/g and 6126 mg/g.

1.3.2.6 Phytosterols

Phytosterols is an important structural component of plant membrane similar in structure to cholesterol, but different in configuration. Recent studies have shown that natural intake of dietary plant sterols can have a positive effect in decreasing serum cholesterol levels, protect

against CVD, and prevent colon cancer (Miettinen *et al.*, 1995; Hendriks *et al.*, 1999; Awad and Fink, 2000). Cereals are the main source of plant sterols, together with vegetable oils, contributing up to 40% of daily intake of plant sterols. Barley is considered a good source of phytosterol although barley's phytosterol level is moderate compared with other major grains (Frølich *et al.*, 2013). Barley grains generally contain phytosterols in both free and bound forms, esterified to fatty acids, phenolic acids, steryl glucosides, or acylated steryl glycosides. The level of esterification varies among varieties and around different parts of barley grain (Liu and Moreau, 2008). Higher levels of phytosterols have been identified in the outer layers of the kernel. The content of sterols in 10 barley varieties studied by Andersson *et al* (2008) ranged between 820 mg/g and 1153 mg/g. Authors analyzed and compared the phytosterol content of major cereal grains with the highest plant sterol content (mean, 955 mg/g) determined in rye, followed by barley (761 mg/g), wheat (690 mg/g), and oat (447 mg/g). As in most cereals, sitosterol is the most abundant sterol form in barley, contributing about 53-61% of total sterols, followed by campesterol (14-20%). Other reported forms of sterol in barley include brassicasterol, stigmasterol, d5-avenasterol, stigmastenol, stigmastadienol, and d7-avenasterol (Lampi *et al.*, 2004).

1.3.2.7 Folates

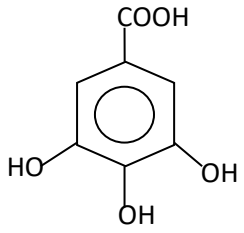
Folate is a group of phytochemicals that represents an essential nutrition component (vitamin B). Folate is capableof performing the same biological activity as folic acid and isinvolved in many metabolic pathways (Romano*et al.*, 1995).Cereals are considered an important source of folate, though little is known about folate in barley. Folate is thought to be unevenlydistributed in barley grain with more concentration aroundthe outer layers than around the endosperm. Edelmann*et al*(2013)studied the distribution of folates in barley grain,which was found to accumulate in the bran and germ tissues of the grain. Andersson *et al* (2008)studied the total folate content of 10 barley genotypes (518-789 mg/g of dry mass), which was found to be higher than that of wheat (323-774 mg/g,n ¼ 130), oat (495-604 mg/g, n ¼ 5) (Shewry *et al.*, 2008), and close to that of rye (574-775 mg/g, n ¼ 10). Edelmann *et al* (2013) also reported a similar folate content of 598-664 mg/g for five hulled barley cultivars studied.

1.3.3 Tannins

Tannins are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in

tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Tannins have been found in a variety of plants utilized as food and feed. These include food grains such as sorghum, millets, barley, dry beans, faba beans, peas, carobs, pigeonpeas, winged beans, and other legumes. Fruits such as apples, bananas, blackberries, cranberries, dates, grapes, hawthornes, peaches, pears, persimmons, plums, raspberries, and strawberries also contain an appreciable quantity of tannins. Likewise, phenolic compounds are present in wines and tea (Thompson *etal*., 1972). Forages such ascrownvetch, lespedeza, lotus, sainfoin, and trefoil are also reported to contain tannins. Tannins can be classified into two categories: hydrolyzable and nonhydrolyzable or condensed tannins. Hydrolyzable tannins contain a central core of polyhydric alcohol such as glucose, and hydroxyl groups, which are esterified either partially or wholly by gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins). After hydrolysis by acids, bases, or certain enzymes, gallotannins yield glucose and gallic acids. The hexahydroxydiphenic acid of ellagitannins undergoes lactonization to produce ellagic acid.

Gallotannin + H_2O ⟶ Glucose + Gallic acid



1.3.4 Bioavailability of polyphenols

Bioavailability is the proportion of the nutrient that is digested, absorbed and metabolized through normal pathways. Generally, aglycones can be absorbed from the small intestine; however, most polyphenols are present in food in the form of esters, glycosides or polymers that cannot be absorbed in native form (D'Archivio *et al.*, 2007).During the course of the absorption, polyphenols undergo extensive modification; infact they are conjugated in the intestinal cells and later in the liver by methylation, sulfation and/or glucuronidation (Day and Williamson, 2001). As a consequence, the forms reaching the blood and tissues are different from those present in food and it is difficult to identify all the metabolites and to evaluate their biological activity (Setchell *et al*., 2003). It is the chemical structure of polyphenols that determines the rateof

absorption and the nature of the metabolites in the plasma. The most common polyphenols in our diet are not necessarily those showing highest concentration of active metabolites in target tissues; consequently, the biological properties of polyphenols greatly differ from one polyphenol to another (Duthie *et al.,* 1998).

Some of the polyphenols are absorbed in the gastro-intestinaltract while others in intestine or other part of the digestive tract. In foods, all flavonoids except flavanols exist in glycosylatedforms (Joseph *et al.,* 2005). The fate of glycosides in the stomach is not clearyet. Most of the glycosides probably resist acid hydrolysis in the stomach and thus arrive intact in the intestine (Gee *et al.,* 1998) where only aglyconesand few glucosides can be absorbed. Experimental studiescarried out in rats (Crespy *et al.,* 2002) showed that the absorption at gastric level ispossible for some flavonoids, such as quercetin, but not for their glycosides. (Passamonti *et al.,* 2005; D'Archivio *et al.,* 2007). Proanthocyanidins differ from most of other plant polyphenols because of their polymeric nature and high molecular weight. This particular feature should limit their absorption through the gut barrier, and oligomers larger than trimers are unlikely to be absorbed in the small intestine in their native forms (Halliwell *et al.,* 2000; Passamonti *et al.,* 2005).

When hydroxycinnamic acids areingestedin the free form, they are rapidly absorbed by the small intestine andare conjugated as the flavonoids (Clifford, 2000). However, these compounds arenaturally esterified in plant products and esterification impairs their absorption because intestinal mucosa, liver and plasma do not possess esterases capable of hydrolyzing chlorogenic acid torelease caffeic acid, and hydrolysis can be performed only by the microflora present in colon (Olthof *et al.,* 2001).Some polyphenols are not absorbed in these locations. These polyphenols reach the colon, where microflora hydrolyze glycosidesinto aglycones and extensively metabolize these aglycones into various aromatic acids.Aglycones are split by the opening of the heterocycle at different points depending on their chemical structure, and thus produce different acids that are further metabolized to derivatives of benzoic acid. After absorption, polyphenols go to several conguation processes, such as: methylation, sulfation and glucuronidation, representing a metabolic detoxicationprocess, common to many xenobiotics, that facilitates their biliary and urinary elimination by increasing their hydrophilicity (Lee *et al.*,2002). Sulfo-transferases catalyze the transferof a sulfate moiety during process of sulphonation. The sulfation occurs mainly in the liver, but the position of sulfation for polyphenols have not been clearly identified yet (Falany, 1997). Glucuronidation occurs in the

intestine and in the liver, and the highest rate of conjugation is observed in the C3-position (Spencer *et al.,* 1999). The conjugation mechanisms are highly efficient and free aglycones are generally either absent, or present in low concentrations in plasma after consumption of nutritional doses (Hollman *et al.,* 2015).

Polyphenol metabolites circulate in the blood bound to proteins; in particular albumin represents the primary protein responsible for the binding (Dangles *et al.,* 2001). Binding to albumin may have consequences for the rate of clearance of metabolites and for their delivery to cells and tissues. It is possible that the cellular uptake of metabolites is proportional to their unbound concentration (D'Archivio *et al.,* 2007;Dufour *et al.,* 2007).Accumulation of polyphenols in the tissues is the most important phase of polyphenol metabolism because this is the concentration which is biologically active for exerting the effects of polyphenols. Studies have shown that the polyphenols are able to penetrate tissues, particularly those in which they are metabolized such as intestine and liver. Excretion of polyphenols with their derivatives occurs through urine and bile. It has been observed that the extensively conjugated metabolites are more likely to be eliminated in bile, whereas small conjugates, such as monosulfates, are preferentially excreted in urine. Urinary excretion percentage is quite high for flavanones from citrus fruit and decreases from isoflavones to flavonols (D'Archivio *et al.,* 2007).

1.3.5Polyphenols and Human health

Epidemiological studies have repeatedly shown an inverse association between the risk of chronic human diseases and the consumption of polyphenolic rich diet (Scalbert *et al.,* 2005; Arts and Hollman, 2005). The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components (Clifford, 2000). It is well established that polyphenol-rich foods and beverages may increase plasma antioxidant capacity. This increase in the antioxidative capacity of plasma following the consumption of polyphenol-rich food may be explained either by the presence of reducing polyphenols and their metabolites in plasma, by their effects upon concentrations of other reducing agents (sparing effects of polyphenols on other endogenous antioxidants), or by their effect on the absorption of pro-oxidative food components, such as iron (Scalbert *et al.,* 2005). Consumption of antioxidants has been associated with reduced levels of oxidative damage to lymphocytic DNA. Similar observations have been made with polyphenol-rich food and beverages indicating the protective effects of polyphenols (Vitrac *et al.,* 2002). There are increasing evidences that as antioxidants, polyphenols may protect cell constituents against

oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress (Pandey *et al.,* 2009; Pandey and Rizvi, 2009).

1.3.5.1 Cardio-Protective effects of polyphenols

Studies demonstrated that consumption of polyphenols limits the incidence of coronary heart diseases (Renaud and de Lorgeril 1992; Dubick and Omaye, 2001; Nardini *et al.,* 2007). Atherosclerosis is a chronic inflammatory disease that develops in lesion-prone regions of medium-sized arteries(Vita, 2005). Polyphenols are potent inhibitors of LDL oxidation and this type of oxidation is considered to be a key mechanism in development of atherosclerosis. Other mechanisms by which polyphenols may be protective against cardiovascular diseases are antioxidant, anti-platelet, anti-inflammatory effects as well as increasing HDL, and improving endothelial function (García-Lafuente *et al.,* 2009). Quercetin, the abundant polyphenol in onion has been shown to be inversely associated with mortality from coronary heart disease by inhibiting the expression of metalloproteinase 1 (MP1), and the disruption of atherosclerotic plaques(Maeda *et al.,*2003). Polyphenols may also exert antithrombotic effects by means of inhibiting platelet aggregation. Polyphenols can improve endothelial dysfunction associated with different risk factors for atherosclerosis before the formation of plaque; its use as a prognostic tool for coronary heart diseases has also been proposed (Schachinger *et al.,* 2002). It has been observed that consumption of black tea about 450 ml increases artery dilation 2 hours after intake and consumption of 240 ml red wine for 30 days countered the endothelial dysfunction induced by a high fat diet (Duffy *et al.,* 2001).

Resveratrol, the wine polyphenol prevents the platelet aggregation via preferential inhibition of cyclooxygenase1(COX 1) activity, which synthesizes thromboxane A2, an inducer of the platelet aggregation and vasoconstrictor (Pirola and Frodjo, 2008). In addition to this, resveratrol is capable of relaxing the isolated arteries and rat aortic rings. The ability to stimulate Ca^{2+} activated K^{+} channels and to enhance nitric oxide signaling in the endothelium are other pathways by which resveratrol exerts vasorelaxant activity (Pirola and Frodjo, 2008; Harikumar and Aggarwal 2008).Studies have shown that resveratrol potentially inhibits the oxidation of the LDL particles via chelating copper or by direct scavenging of the free radicals. Resveratrol is the active compound in red wine which is attributed for “French Paradox”, the low incidence of CVD despite the intake of high-fat diet and smoking among French (Peters *et al.,* 2001; Cucciolla *et al.,* 2007; Shakibaei *et al.,* 2009).

1.3.5.2 Anti-Cancer effects of polyphenols

Effect of polyphenols on human cancer cell lines, is most often protective and induce a reduction of the number of tumors or of their growth (Yang *et al.,* 2001). These effects have been observed at various sites, including mouth, stomach, duodenum, colon, liver, lung, mammary gland or skin. Polyphenols such as, quercetin, catechins, isoflavones, lignans, flavanones, ellagic acid, red wine polyphenols, resveratrol and curcumin have been tested; all of them showed protective effects in some models although their mechanisms of action were found to be different (Johnson *et al.,* 1994).Into the three major stages of carcinogenesis: initiation, promotion and progression. Cells initiated can undergo transformation to malignancy if promotion and progression follow. Promotion, on the other hand, is affected by factors that do not alter DNA sequences and involves the selection and clonal expansion of initiated cells (Cao *et al.,* 1998). Mechanisms ofchemo-preventiveeffect of polyphenols, include estrogenic/antiestrogenic activity, antiproliferation, induction of cell cycle arrest or apoptosis, prevention of oxidation, induction of detoxification enzymes etc (Athar *et al.,* 2007).Polyphenols influence the metabolism of pro-carcinogens by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens. (Khan and Mukhtar, 2008).

Theaflavins and thearubigins, the abundant polyphenols in black tea have also been shown to possess strong anticancer property. Black tea polyphenols were found to inhibit proliferation and increase apoptosis in Du 145 prostate carcinoma cells. Black tea polyphenol addition was found to block IGF-1 induced progression of cells into S phase of cell cycle at a dose of 40 mg/ml in prostate carcinoma cells (Sharma and Rao *et al.,* 2009). Quercetin has also been reported to possess anticancer property against benzo(a)pyrene induced lung carcinogenesis in mice, an effect attrtibuted to its free radical scavenging activity (Kamaraj *et al.,* 2007).

1.3.5.3 Anti-Diabetic effects of polyphenols

Studies have shown that several physiological parameters of the body get altered in the diabetic conditions (Rizvi and Zaid, 2001;Rizvi and Zaid, 2005). Long term effects of diabetes include progressive development of specific complements such as retinopathy, which affects eyes and lead to blindness; nephropathy in which the renal functions are altered or disturbed and neuropathy which is associated with the risks of amputations, foot ulcers and features of autonomic disturbance including sexual dysfunctions(Rizvi and Zaid 2001; Rizvi *et al.,* 2005).Polyphenols may affect glycemia through different mechanisms, including the inhibition

of glucose absorption in the gut or of its uptake by peripheral tissues. The hypoglycemic effects of diacetylated anthocyanins at a 10 mg/kg diet dosage were observed with maltose as a glucose source, but not with sucrose or glucose (Matsui, 2002). This suggests that these effects are due to an inhibition of α-glucosidase in the gut mucosa. Inhibition of α-amylase and sucrase in rats by catechin at a dose of about 50 mg/kg diet or higher was also observed.

Individual polyphenols, such as (+)catechin, (-)epicatechin, (-)epigallocatechin, epicatechin gallate, isoflavones from soyabeans, tannic acid, glycyrrhizin from licorice root, chlorogenic acid and saponins also decrease S-Glut-1 mediated intestinal transport of glucose. Saponins additionally delay the transfer of glucose from stomach to the small intestine (Dembinska-Kiec *et al.,* 2008). Many mechanisms have been proposed to explain the anti-diabetic action of this stilbene, modulation of SIRT1 is one of them which improves whole-body glucose homeostasis and insulin sensitivity in diabetic rats (Milne *et al.,* 2007; Harikumar and Aggarwal, 2008). It is reported that in cultured LLC-PK1 cells, high glucose induced cytotoxicity and oxidative stress was inhibited by grape seed polyphenols. Treatment with resveratrol also decreased insulin secretion and delayed theonset of insulin resistance. A possible mechanism was thought to be related to the inhibition of K + ATP and K + V channel in beta cells (Chen *et al.,* 2012).

A recent study shows that quercetin has ability to protect the alterations in diabetic patients during oxidative stress. Quercetin significantly protected the lipid peroxidation and inhibition antioxidant system in diabetics(Rizvi and Mishra, 2009). A study performed by Lee*et al*.,(2009) showed that polyphenols present in the extracts from *Hibiscus sabdariffa* attenuate diabetic nephropathy including pathology, serum lipid profile and oxidative markers in kidney.

1.3.5.4 Anti-Aging effects of polyphenols.

Among many theories purposed for explaining the mechanism of aging, free radical/oxidative stress theory is one of the most accepted one(Harman, 2006). A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during the aging process as the efficiency of antioxidative and repair mechanisms decrease(Rizvi and Maurya, 2007). Antioxidant capacity of the plasma is related to dietary intake of antioxidants. Intake of antioxidant rich diet is effective in reducing the deleterious effects of aging and behavior. Subset of the flavonoids known as anthocyanins, are particularly abundant in brightly colored fruits such as berry fruits and concord grapes and grape seeds. Anthocyanins have been shown to have potent antioxidant/anti-inflammatory activities, as well as to inhibit

lipid peroxidation and the inflammatory mediators - cyclo-oxygenase (COX)-1 and -2 (Seeram *et al.,* 2003).

Fruit and vegetable extracts that have high levels of flavonoids also display high total antioxidant activity such as spinach, strawberries and blueberries. It is reported that the dietary supplementations (for 8 weeks) with spinach, strawberry or blueberry extracts in a control diet were also effective in reversing age-related deficits in brain and behavioral function in aged rat. A recent study demonstrates that the tea catechins carry strong anti-aging activity and consuming green-tea rich in these catechins, may delay the onset of aging(Maurya and Rizvi, 2008). Polyphenols are also beneficial in ameliorating the adverse effects of the aging on nervous system or brain. Paramount importance for the relevance of food polyphenols in the protection of the aging brain is the ability of these compounds to cross the blood-brain barrier (BBB), which tightly controls the influx in the brain of metabolites and nutrients as well as of drugs. Resveratrol has been found to consistently prolong the life span; its action is linked to an event called caloric restriction or partial food deprivation (Harikumar and Aggarwal, 2008).

1.3.5.5 Neuro-Protective Effects of polyphenols

Oxidative stress and damage to brain macromolecules is an important process in neurodegenerative diseases. Alzheimer's disease is one of the most common occurring neuro-disorders affecting up to 18 million people worldwide (Dai *et al.,* 2006). It was observed that the people drinking three to four glasses of beer per day had 60% decreased incidence of dementia and Alzheimer's disease compared to abstainers (Scarmeas *et al.,* 2007). Resveratrol scavenges O2 - and OH• in vitro, as well as lipid hydroperoxyl free radicals, this efficient antioxidant activity is probably involved in the beneficial effect of the moderate consumption of beer against dementia in the elderly. Resveratrol inhibits nuclear factor κB signaling and thus gives protection against microglia dependent β-amyloid toxicity in a model of Alzheimer's disease and this activity is related with the activation of the SIRT-1(Markus and Morris, 2008). Polyphenols from fruits and vegetables seem to be invaluable potential agents in neuro protection by virtue of their ability to influence and modulate several cellular processes such as signaling, proliferation, apoptosis, redox balance and differentiation (Singh *et al.,* 2008).

Administration of polyphenols provide protective effects against Parkinson's disease,a neurological disorder characterized by degeneration of dopaminergic neurons in the *substantia nigra zona compacta* (Aquilano *et al.,* 2008). Nutritional studies have linked the consumption of

green tea to the reduced risk of developing Parkinson's disease. In animal models epigallocatechin gallate (EGCG) has been shown to exert a protective role against the neurotoxin MPTP (N-methyl-4- phenyl-1,2,3,6-tetrahydropyridine), an inducer of a Parkinson's like disease, either by competitively inhibiting the uptake of the drug, due to molecular similarity or by scavenging MPTP-mediated radical formation. (Rossi *et al.,* 2008). The therapeutic role of catechins in Parkinson's disease is also due to their ability to chelate iron. This property contributes to their antioxidant activity by preventing redox-active transition metal from catalyzing free radicalformation (Aquilano *et al.,* 2008). Epidemiological evidence that polyphenols may protect against obstructive lung disease is the product of studies that have documented negative associations of apple intake with asthma prevalence and incidence, and a positive association with lung function (Tabak *et al.,* 2001; Woods *et al.,* 2003). Animal study shows that, when applied orally or topically, polyphenols present in tea ameliorate adverse skin reactions following UV exposure, including skin damage, erythema and lipid peroxidation (Kim *et al.,* 2001).

1.4 Residual sugar in beer

Residual sugar is the sugar left unfermented in beer or wine, measured in grams/litre (g/l). Sugar is a building block of carbohydrates and it is naturally found in many foods such as fruit, milk and grain. Another kind of sugar is added sugar which can be founded in flavored yogurt, sweetened beverages, baked goods and cereals,and it is used widely in industries. Sugar is a natural and nontoxic, sweet testing, water soluble crystalline carbohydrates, and every 1 gram of sugar provides the body with 4 kilocalories. In production industries, sugars are defined as caloric sweeteners that are added to foods during processing. Most times they are referred to as added sugars. The mainsource of sugar is the beet sugar orcane sugar; also there are other several sources such as honey,cornsyrup, fruits, and vegetablesetc. (Erdal *et al.*, 2007). The primary functionof sugar in food products is to provide sweetness and energy,in addition, sugar plays a very important role in preservation,fermentation, color and texture (Hu, 2013).

Sugarsadded to foods during processing are most times referred to as added sugars. Examples include all types of syrups, brown sugar, cane sugar, corn sweetener, dextrose, fructose, glucose, granulated sugar, high-fructose corn syrup, honey, invert sugar, lactose, malt syrup, maltose, molasses, raw sugar, sucrose, trehalose, and turbinado sugar. Additionally, fruit juice concentrates used in foods without further dilution are also considered as added sugars. There is

also a definition for sugars that are not added sugars. Although the consumption of sugar has been reported to be associated with detrimental health conditions, such as dental caries, (Armfield *et al.,* 2013) asthma, (Park *et al.,* 2013) obesity, (Ma *et al.,* 2016) altered lipid profiles, (Kell *et al.,* 2014) and elevated blood pressure; it still determines the taste of many foods and beverages.

1.4.1 Carbohydrates

Carbohydrates are one of the most abundant biomoleculeson Earth, sugar and starch are the essential part of food in mostplaces of the world. Carbohydrates can be present in large amounts in beer,since it is made from barley. The main roles of carbohydrate are asenergy stores, fuels, metabolic intermediates and part of thestructural framework of RNA and DNA. Carbohydrates arealdehydes, ketones with many hydroxyl groups, consist ofcarbon, oxygen and hydrogen, the empirical formula is $(CH_2O)n$ and are classified as monosaccharide (which cannot behydrolysed into smaller sub-units), disaccharide andpolysaccharides (Rosa *et al.,* 2009). The monosaccharides, depending on the position of thecarbonyl group, are classified as either aldoses or ketoses.Tetroses, pentoses, hexoses and heptoses have four, five, sixand seven carbon atoms respectively. Monosaccharides, arethe building blocks of carbohydrate chemistry. The commonsix-carbon sugars (hexoses) are D-glucose, D-fructose, andD-galactose. They all are aldohexoses, except D-fructose,which is a ketohexose (Rosa *et al.,* 2009).

Absorption of monosaccharides occurs in the smallintestine without further chemical breakdown. intestinal villi is the site of glucose absorption via co-transport with sodiumions; it then enters the capillary blood for eventual transport tothe liver.Polysaccharides,oligosaccharides and disaccharides are hydrolyzed by enzymes prior to their absorption in the smallintestine. Salivary amylase which present in the saliva, breaksdown polysaccharides into oligosaccharides. Once in thestomach, the acidity deactivates this enzyme (Kosova *et al.,* 2013). Thepolysaccharides that survived the salivary amylase are furtherbroken down in the intestine by pancreatic amylase. Intestinalbrush border enzymes further hydrolyze oligosaccharides anddisaccharides into their constituent monosaccharides. Suchenzymes include dextrinase, glucoamylase, maltase, sucraseand lactase. Chemical digestion ends in the small intestinebecause the colon does not secrete digestive enzymes (Margaret *et al.,* 2018).

1.4.1.1 Classification of carbohydrate

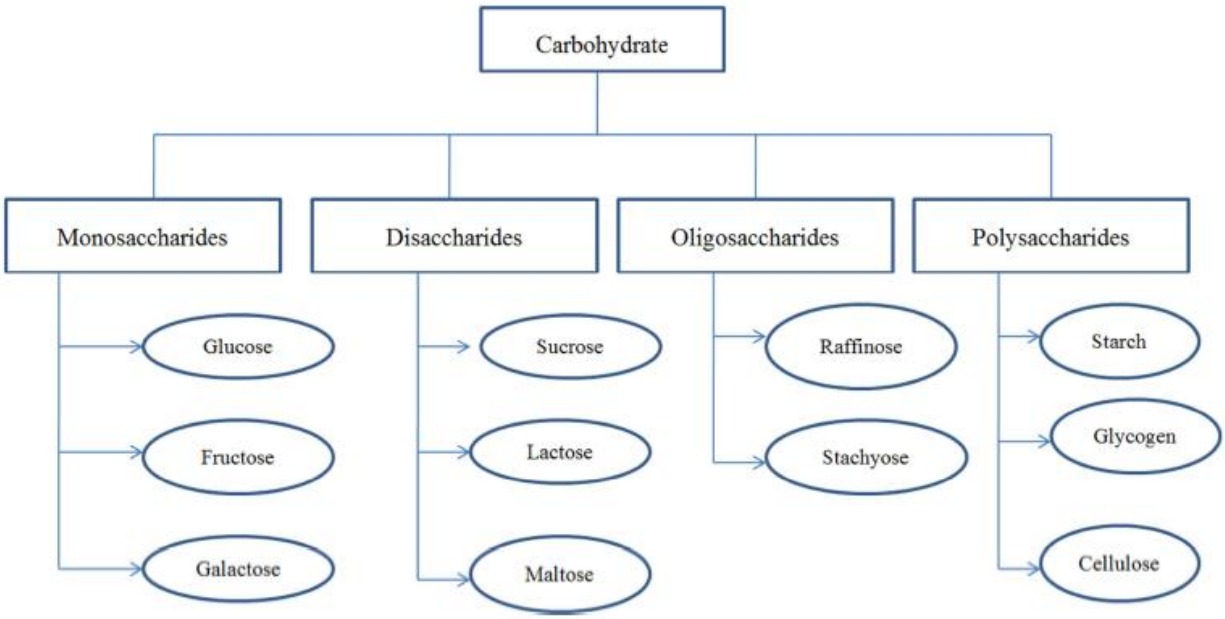


Figure 5. Classification of carbohydrate (Margaret *et al.,* 2018).

In beer production, sugar forms the raw material on which fermentation is based, and thus it is necessary that accurate determinations of the individual components of mixtures of sugars be made at all stages of beer production and processing. Beer is a very complex mixture, major beer components are water, ethanol and carbohydrates comprising fermentable sugars (i.e. fructose, glucose, maltose and maltotriose) as well as glucose oligosaccharides and arabinoxylans (Hughes and Baxter, 2001; Caballero *et al.*, 2003; Briggs *et al*., 2004). Monitoring of beer carbohydrate composition may therefore be an important tool for modern brewing technology, particularly in the selection of raw materials and yeast strains, product development and quality control. Quantitative evaluation of malto-oligosaccharides provides a useful control of the complex enzymatic system in beer brewing, particularly when changes in procedure are con-templated. In this respect it is important to control not only the total amount of fermentable carbohydrates formed but also the relative amounts of the different sugars.

1.4.2 Functional Properties of Sugar inFood

In addition to main role of sugars in providing sweet taste,there are large functions of sugars in food.

1.4.2.1 Sweetness

The most apparent sensory property of sugars such asglucose, fructose, and sucrose is their sweetness, Lactose(milk sugar) is the least sweet, whereas fructose is the sweetestsugar. Sugars are used as sweeteners in many kinds of foodproducts (Gwak*et al.*, 2012).

1.4.2.2 Flavour

Sugar contributes tothe flavor of food by interacting with other components toimprove or lessen certain flavors. By adding a small amount ofsugar to cooked vegetables and meat enhance the food'snatural flavors, without making them taste sweet (Cerny and Davidek, 2003). In sourapplications such as beverages, jams and marmalades whichall mixes of sweet and sour components, it is important tocreate a good balance between sourness and sweetness, whichis often achieved by adding a mix of sugar and citric acid. Inbitter applications, sugar is often used to moderate or disguisethe bitterness (chocolate and coffee) (Murray, 2009).

1.4.2.3 Fermentation

Fermentation of sugar is carried outby yeasts in anaerobicconditions, and carbon dioxide is released as product. This process is veryimportant for bread making, beer and wine. In beer making,sugar plays important roles (in addition to taste), by determing the extent of fermentation through the formation of carbon dioxide(Vaclavik and Christian, 2014).

1.5 Rationalefor the study

There are many beer brands that are available in Nigeria and different people tend to prefer one brand over the other. Most drinkers prefer one brand of beer over the other because of the taste. Thus, it is important to assess the quantity of two classes of compounds, namely polyphenols and sugars, which are known to contribute to the taste of beer.

1.6 Aim and Objectives of the study

1.6.1 Aim of the study

This study was aimed at quantifying the polyphenolic and residual sugar constituents of various brands of beer.

1.6.2 Objectives of the study

Specific objectives of the study include:

i. to quantify the total phenolics (TP) content of different beer samples
ii. to quantify the total tannin content of the beer samples
iii. to analyze the concentration of total anthocyanin content in the samples
iv. to quantify theleucoanthocyanin content of beer samples
v. to quantify the residual sugar in beer samples.
vi. to physicallyanalyze the various brands of beer

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Beer samples

A total of fourteen (14) beer samples were randomly selected from different vendor outlet within and outside Nsukka metropolis. The samples were collected in a clean sterilized sample bottles and were taken to the Department of biochemistry, University of Nigeria, Nsukka, Enugu state where they were properly stored at -4^0C for various analyses.

Plate 1: Beer samples

2.1.2 Chemicals and Reagents

All chemicals used in this study were of analytical grade and products of Sigma Aldrich, (USA), British Drug House (BDH) England, Burgoyne, (India), Harkin and Williams, (England), Qualikems (India), Fluka (Germany), May and Baker, (England). Reagents used for all the experiments were commercial kits and products of Randox, (USA) and Teco (TC), (USA) these include: methanol, HCl, complex cyanoferric acid, sulphuric acid, ferric chloride, phenolic folin ciocateau, peroxide reagents, buffer salts, acetone, sodium carbonate, Di nitrosalycyclic acid, sodium tartarate.

2.1.3 Instruments and Equipment

Water bath (Gallenkamp England), Refrigerator (Kelvinator Germany), Colorimeter (E1 312 Model, Japan), Microlux micro pipette, Centrifuge (4000rpm Abman), Analytical Chemical Balance (Ohaus Corp AR 3130, China), pH meter, UV-Vis Spectrophotometer (Jenway 6305, China), Pasteur pipette, Fluorescent tube and Aluminum foil.

2.2 Methods

2.2.1 Experimental Design

The present study was carried out in five (5) different stages:

i. sampling of the different beer liquor samples
ii. determination of physical properties of the beer liquor
iii. determination of the polyphenol content of the different beer liquor
iv. determination of the tannic acid content of the different beer liquor
v. determination of the pro/anthocyanin content of the different beer liquor
vi. determination of the residual sugar content of the different beer liqoure

2.2.2 Determination of total phenolic content in Beer

2.2.2.1 Principles

Total phenolics were determined using Folin-Ciocalteau Reagent (FCR) as described by A.O.A.C. (1997), with slight modifications. FCR consist of a yellow acidic solution containing complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. Dissociation of a phenolic proton in a basic medium leads to a phenolate anion, which reduces

FCR forming a blue coloured molybdenum oxide whose colour intensity is directly proportional to the phenolic contents. 1ml of each of the beer samples diluted with 7 ml of distilled water in test tubes was mixed with 500 µl of Folin-Ciocalteu reagent (diluted 10-fold in dH_2O) and allowed to stand at 22 ^oC for 3 min; 1000 µl of Na_2CO_3 (60 g/l) solution was then added to the mixture and made up to 10 ml with distilled water. After 60 min the absorbance was measured at 725 nm. Results were expressed as gallic acid equivalents.

2.2.3 Determination of total tannin content in Beer

Tannin content in each sample was determined using insoluble Potassium hexacyanoferrate, which binds tannins as described by Butler and Price. (1977). 3 ml of 0.1M ferric chloride in 0.1 N HCl was added to 15 ml of the beer samples in test tubes. This was followed by immediate addition of 3ml of 0.008 M potassium hexa cyanoferrate, the mixture was allowed to stand for 10 minutes at room temperature, thereafter absorbance was read off at 720 nm.

2.2.4 Determination of Anthocyanin content in Beer

The method of Swain and Hills (1959) was used for the determination of anthocyanin contents of the beer samples.1ml of the sample was placed in a test tube. The content of the tube was mixed with three millilitres of 80% (v/v) hydrochloric acid in methanol. The solution was diluted with 1.0 ml of peroxide reagent (1ml of 30% H_2O_2in 9ml of methanolic hydrochloric acid (5:1, v/v)). The tube was left for 15 minutes in the dark and the absorbance of the solution was measured in a 1-cm cell at 525nm. The blank was prepared in a second tube by mixing the reagents without the sample.

2.2.5 Determination of leucoanthocyanin content in Beer

The leucoanthocyanin contents of the sample was estimated using the method of Swain and Hills (1959). 1.0 ml of the sample in a test tube was mixed with 10 ml of leucoanthocyanin reagent, and the tube was shaken vigorously until the contents became homogenous. The tube was heated in a water–bath at 96^0C for 40 minutes. At the end of heating, the tube was cooled, and thereafter, the absorbance was determined in a 1-cm cuvette at 550nm. The blank formulated in the same procedure but not heated.

2.2.6 Estimation of Residual sugar content

This was achieved by measuring the glucose content (residual) of the beer samples using a modification of the 3, 5-dinitrosalicylic acid (DNS) reagent assay method described by Miller (1959). The reaction mixture contained 0.5ml of each of the beer samples in 0.05M sodium acetate buffer of pH 5.5, the solution was incubated for 1 hour. 1ml of DNS reagent was added and the mixture was boiled in a boiling water bath for 10minutes. The reaction was stopped by the addition of 1ml of Rochelle salt solution and the reaction mixture was made up to 4ml by addition of 1ml of distilled water. The reaction mixture was allowed to cool and then the absorbance read at 540 nm.

2.2.7 Statistical Analysis

Data obtained were reported as Mean ± Standard Deviation where appropriate. One-way Analysis of Variance (ANOVA) and correlation analyses were used to analyze the experimental results using The Statistical Product and Services (SPSS) version 21. The level of significance was observed at $P < 0.05$.

CHAPTER THREE

RESULTS

3.1 Estimation of the pH Values of the Various Brands of Beer

Table 1 shows the pH values of the 14 brands of beer. Their pH values were found to range between 4.05 – 5.25, indicating that all the brands analyzed were acidic. Beer samples 5, 10 and 14, among the brands analyzed, were found to be more basic; while sample 13 was the most acidic.

3.2 Quantification of the Total Phenolic Content of different Brands of Beer.

Table 2 shows the concentration of total phenolic compounds present in various brands of beer using Folin Ciocalteu Reagent, (expressed as gallic acid equivalents), which is based on the reduction of phosphotungstate – phosphomolybdate complex by phenolic compounds to blue reaction products. Heineken and tiger recorded the highest total phenolic content, 87.91 and 87.86 mM GAE respectively.

3.3 Quantification of Total Tannin Content of the Beer Samples.

Table 3 shows the concentration of total tannin present in the beer brands using Potassium hexacyanoferrate method. The total tannin concentration was in the range of 1.17 – 2.02 mg/ml tannic acid equivalents, with sample 6 being the most tannic. The lowest concentration of tannic acid was recorded in samples 10 and 5.

3.4 Quantification of total anthocyanin in different beer brands

Table 4 shows the concentration of total anthocyanin in different beer brands using the method described by Swain and Hills (1959). The brands analyzed varied, but not widely in their concentration of anthocyanin from each other. The quantity of anthocyanin in the samples ranged from 3.52 – 6.38 (%), with sample 8 recording highest anthocyanin content and sample 14 having the lowest value among the brands analyzed.

Table 1. Estimation of pH values of the brands of beer.

Beer samples	pH
Sample 1	4.80 ± 0.10
Sample 2	4.90 ± 0.10
Sample 3	4.85 ± 0.22
Sample 4	4.75 ± 0.22
Sample 5	5.25 ± 0.22
Sample 6	4.60 ± 0.31
Sample 7	5.00 ± 0.31
Sample 8	4.60 ± 0.31
Sample 9	5.20 ± 0.00
Sample 10	5.25 ± 0.22
Sample 11	4.85 ± 0.22
Sample 12	4.70 ± 0.00
Sample 13	4.05 ± 0.22
Sample 14	5.05 ± 0.22

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

Table 2. Total phenolic content of various beer brands

Beer samples	Total phenolics (mM)
Sample 1	72.10 ± 20.48
Sample 2	74.00 ± 12.47
Sample 3	85.41 ± 4.60
Sample 4	83.38 ± 16.68
Sample 5	54.37 ± 11.14
Sample 6	74.32 ± 34.82
Sample 7	87.91 ± 11.48
Sample 8	61.12 ± 3.47
Sample 9	66.07 ± 0.60
Sample 10	78.06 ± 18.76
Sample 11	83.15 ± 5.26
Sample 12	60.22 ± 3.40
Sample 13	35.60 ± 7.67
Sample 14	87.86 ± 3.14

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

Table 3. Quantification of the total tannin content of beer brands

Beer samples	Total tannin content (mg/ml)
Sample 1	1.30 ± 0.11
Sample 2	1.57 ± 0.50
Sample 3	1.81 ± 0.10
Sample 4	1.58 ± 0.76
Sample 5	1.17 ± 0.38
Sample 6	2.02 ± 0.11
Sample 7	1.43 ± 0.48
Sample 8	1.62 ± 0.11
Sample 9	1.39 ± 0.04
Sample 10	1.17 ± 0.17
Sample 11	1.41 ± 0.30
Sample 12	1.92 ± 0.04
Sample 13	1.28 ± 0.04
Sample 14	1.85 ± 0.52

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

Table 4. Quantification of anthocyanin content in various beer brands

Beer samples	Anthocyanin (%)
Sample 1	5.16 ± 1.73
Sample 2	4.43 ± 0.18
Sample 3	4.49 ± 0.18
Sample 4	4.86 ± 0.69
Sample 5	5.71 ± 0.80
Sample 6	4.57 ± 0.15
Sample 7	4.66 ± 0.51
Sample 8	5.38 ± 1.41
Sample 9	4.19 ± 0.35
Sample 10	5.15 ± 1.23
Sample 11	4.11 ± 0.49
Sample 12	5.28 ± 0.25
Sample 13	5.22 ± 0.66
Sample 14	4.08 ± 0.78

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

3.5 Quantification of Total Leucoanthocyanin in Various Beer Brands

Table 5 shows the concentration of leucoanthocyanin in different brands of beer. The leucoanthocyanin was in the range of 1.3 – 5.8, implying that all the beer brands analyzed contained leucoanthocyanin. Samples 5 and 11 recorded higher values, having 4.88 and 5.1 respectively. Among the brands analyzed, sample 14 had the lowest value of total leucoanthocyanin at 3.51 %.

3.6 Quantification of Residual Sugar Constituent in Various Brands of Beer

Table 6 shows the concentration of residual sugar present in different brands of beer, using a method involving 2, 3 – dinitrosalicylic acid (DNS). The results were expressed as glucose equivalents. The residual sugar concentration was in the range of 2.71 – 7.26 Mm/l with sample 4 recording the highest concentration. The lowest concentration of residual sugar was observed in sample 14.

Table 5. Quantification of total leucoanthocyanin in various brands of beer

Beer samples	Leucoanthocyanin (%)
Sample 1	3.99 ± 0.55
Sample 2	4.10 ± 0.35
Sample 3	3.92 ± 0.07
Sample 4	3.74 ± 0.33
Sample 5	5.01 ± 0.18
Sample 6	4.58 ± 0.23
Sample 7	4.64 ± 0.90
Sample 8	4.80 ± 0.47
Sample 9	3.79 ± 0.10
Sample 10	3.89 ± 0.44
Sample 11	5.01 ± 0.13
Sample 12	4.58 ± 0.46
Sample 13	4.77 ± 0.64
Sample 14	4.09 ± 0.83

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

Table 6. Residual sugar constituents of different brands of beer

Sample	Concentration (mM)
Sample 1	6.42 ± 2.16
Sample 2	5.73± 0.57
Sample 3	6.43 ± 0.81
Sample 4	7.26± 2.13
Sample 5	6.43± 1.02
Sample 6	6.09 ± 2.54
Sample 7	4.35 ± 0.85
Sample 8	6.18 ± 0.97
Sample 9	5.31 ± 0.38
Sample 10	7.58 ± 0.19
Sample 11	6.92 ± 0.72
Sample 12	7.35 ± 1.51
Sample 13	5.71± 1.79
Sample 14	2.71 ± 1.02

Results are expressed in Means ± Standard Deviation of the duplicate determination (n=2)

CHAPTER FOUR

DISCUSS`ION

The quantification of the phenolic content, using Folin – Ciocalteu Reagent (FCR) revealed that the brands analyzed contained phenolic compounds in wide variation, ranging from 35.59 mg/l – 87.91 mg/l Gallic Acid Equivalents (GAE).The highest concentrationof phenolics wasrecorded in sample 7 while sample 13 had the least value among the analyzed samples.The variation in the phenolic content of the beer samplescould be attributed to differencesin the degree of maturation of the grains used for malting (Butler, 1982). Also, the levels of nutrients in the soils on which the grains were grown is capable of affecting the phenolic concentration (Van Sumerre *eta1*.,1975).Other researchers who quantified phenolics before now obtained similar results.Oladokun *et al.,* (2016) worked on Czech Lager beerand reported values of total phenolics ranging from 74 – 256 mg/l GAE. The results of this present study were similar to their findings, because they were of the same range.Dvorakova *et al.*,(2006) also quantified beer polyphenols and reported 70 -240 mg/l GAEvalues, which the results of this present study also support. However, other authors who worked on beer polyphenols reported higher concentrations of total phenolic contents in mg/lGAE:Ng and Mocek, (1973) reported 150-350mg/l; Berhanu (2014) reported 270–600 mg/l GAE; and Abdoul-latif *et al*., (2012) recorded concentrations of 206–374 mg/l GAE. These variations could be most likely due to the highly varied brewing techniques and ingredients employed in the industries, grain variety, inconsistency in the preparation processes, geographical locations of the grains or the extent of hops boiling.

Total tannin content of the beer brands estimated using insoluble Potassium hexacyanoferrate revealed close range of tannin content across the samples. The total tannin content ranged from 1.17mg/ml (in sample 10) – 2.02mg/ml (in sample 6). Tinkilic and Uyanik (2001) reported tannin concentrations of beer samples to range from 56.1 – 78.23 ug/ml. The location and type of soil has been reported to have an effect on the level of tannin in the grain and that clay soil promotes tannin content of grains (Clifford and Ramirez-Martinez, 1991). Also, there is an established relationship

between high tannin content in plants and a deficiency of soil nitrogen and phosphorous (Davies *etal*.,1964).Tannins are sometimes added to drinks to produce astringency, though this is not acceptable to some brewmasters. According to Nwanguma and Eze (1996), concentrations of polyphenols in grain could increase during germination up to 96h. This increase observed during

steeping may be due to both de *novo* synthesis and polymerisation and makes up for the polyphenols that may have leached into the steep liquor. Furthermore, the variation could also be dependent on the type and level of individual phenolic compounds present. This is due to the fact that different plants contain different types of phenolic compounds (Lo´pez-Laredo *et al*., 2012; Gaivelyte *et al*., 2014; Brahmi *et al.,* 2014). Tropical and near tropical regions of the world produce grains containing high anthocyanin and phenolic content. It is often erroneously assumed that the higher the temperature of a place, the more pigmented will be the grains of the area. However, it is important to know that plants use radiation available to them and not the heat (Mehari *et al.*, 2016; Xu *et al*., 2016).Tannins, like phenolics, in beer have their main source as the malt and hops. To induce astringency, some trained brewmasters introduce tannins into beer (McRae and Kennedy, 2011). In lager beers, tannins induce haze formation by precipitating proteins present in the beer, resulting to turbidity at low temperature.

Residual sugar content, which was quantified using dinitrosalicylic acid (DNS) revealed varying concentrations of sugar in beer brands ranging from 2.71 mM – 7.57mM.Sample 10 recorded highest concentration of sugar in beer, while the least concentration was found in sample 7. Earlier researchers who quantified leftover sugar in beer samples also recorded similar results with slight differences. Opoku *et al.*,(2015) reported sugar concentration in beer in the range of 15.20 – 34.58 g/l; Ayalew and Shibru, (2018) reported 13.4 – 25.8 g/l. The variations in the results obtained could be as a result of the extent of fermentation and/or the difference in the level of yeast used. From this present study, the order of the beer based on the total sugar contents (in mM) was sample 10 > sample 12 > sample 4 > sample 11 >samples 3 and 5 > sample 1 > sample 8 > sample 6 > sample 2 > sample 13 > sample 9 > 14. Samples 10 and 12 were found to be the leading ones. In addition to the possible causes of variation, the composition of the raw materials, preparation process, and fermentation time mentioned earlier are the crucial factors for the difference in the level of sugars in the fermented beverages.

Tables 4 and 5 show the concentrations of anthocyanin and leucoanthocyanin in beer samples. Anthocyanin concentration ranged from 4.08 – 5.71 (%), while leucoanthocyanin concentrations ranged from 3.74 – 5.01 (%).From the results, the anthocyanin content of the brands varied with some containing higher values than others. During the growth cycle of the grain, the degree of exposure to sunlight will affect the concentration of phenolics especially anthocyanins. Generally, the anthocyanin proportion in alcoholic beverages depends on various factors, such as their structure, the grain variety, and the growing conditions such as cereal farming practices and

the regional weather characteristics (Ferrari *et al.*, 2011), solution composition, pH value of the liquor, storage temperature with time, oxidation status, light exposure, the presence of other substances such as ascorbic acid, sugar sulfites, cofactors and metallic ion (Jackson, 2008).

4.2 Conclusion

Beer being one of the world's most consumed alcoholic beverages, has been proved to contain rich nutrients such as carbohydrates, amino acids, minerals, vitamins, and other compounds such as polyphenols. The results of the present study indicate the amounts of different polyphenolic constituents and residual sugar of different varieties of commercial beer. The presence of these phytochemicals and nutrients in beer could be attributed to a variety of health benefits and decreased overall mortality, predominantly from coronary diseases like cardiovascular disease risk (CVD) found in moderate drinkers.

4.3 Contribution to Knowledge

The quantification of total phenolics, tannin, anthocyanin, leucoanthocyanin and residual sugar which represent the focus of this study provide more information about how beers differ in terms of the presence of compounds that contribute to the taste of the beer. The assessment of phenolic content of the beer samples can also provide information to the consumers about the quality and health benefits of the purchased beer. The sugar content also contribute to caloric value of the beer.

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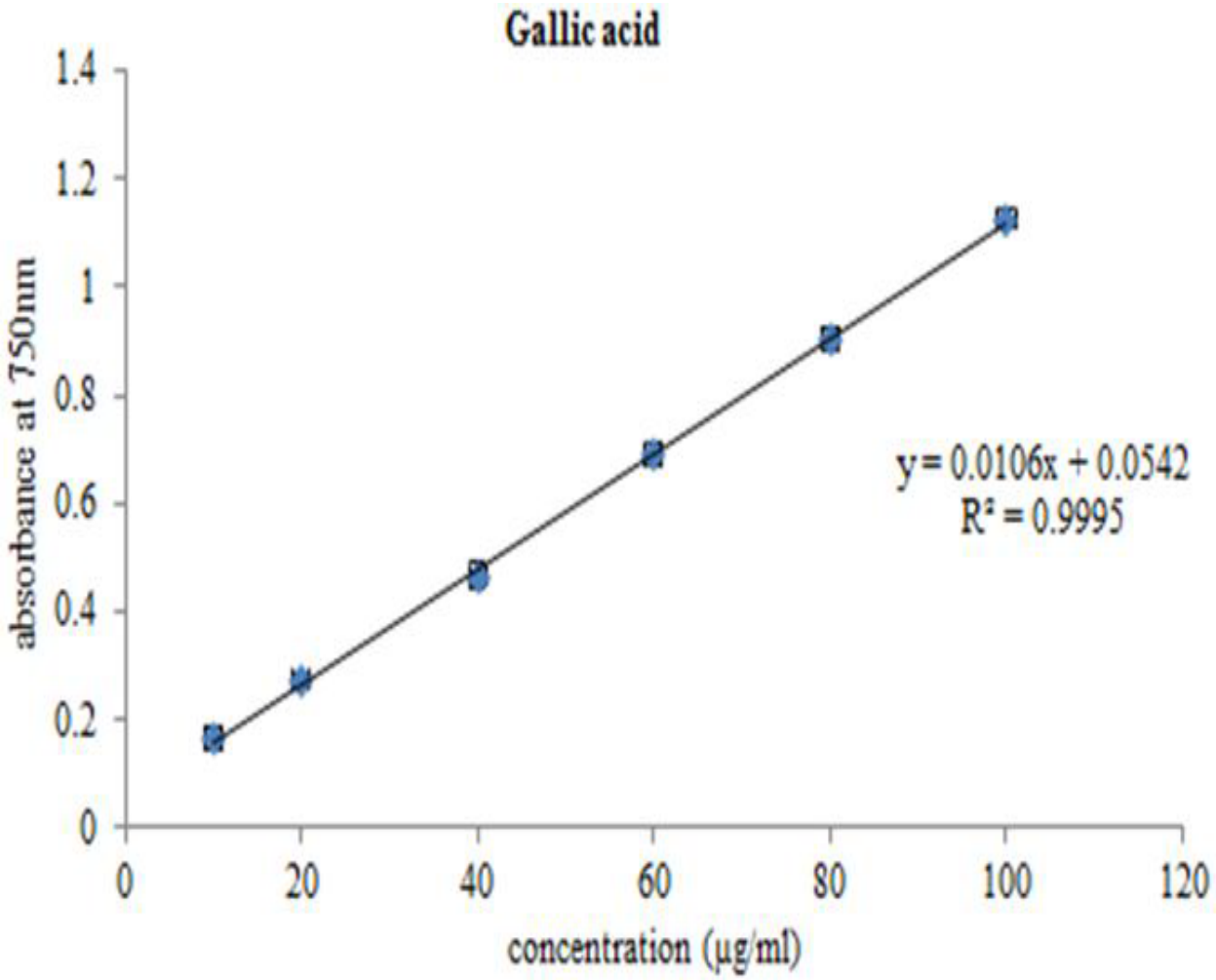
APPENDICES

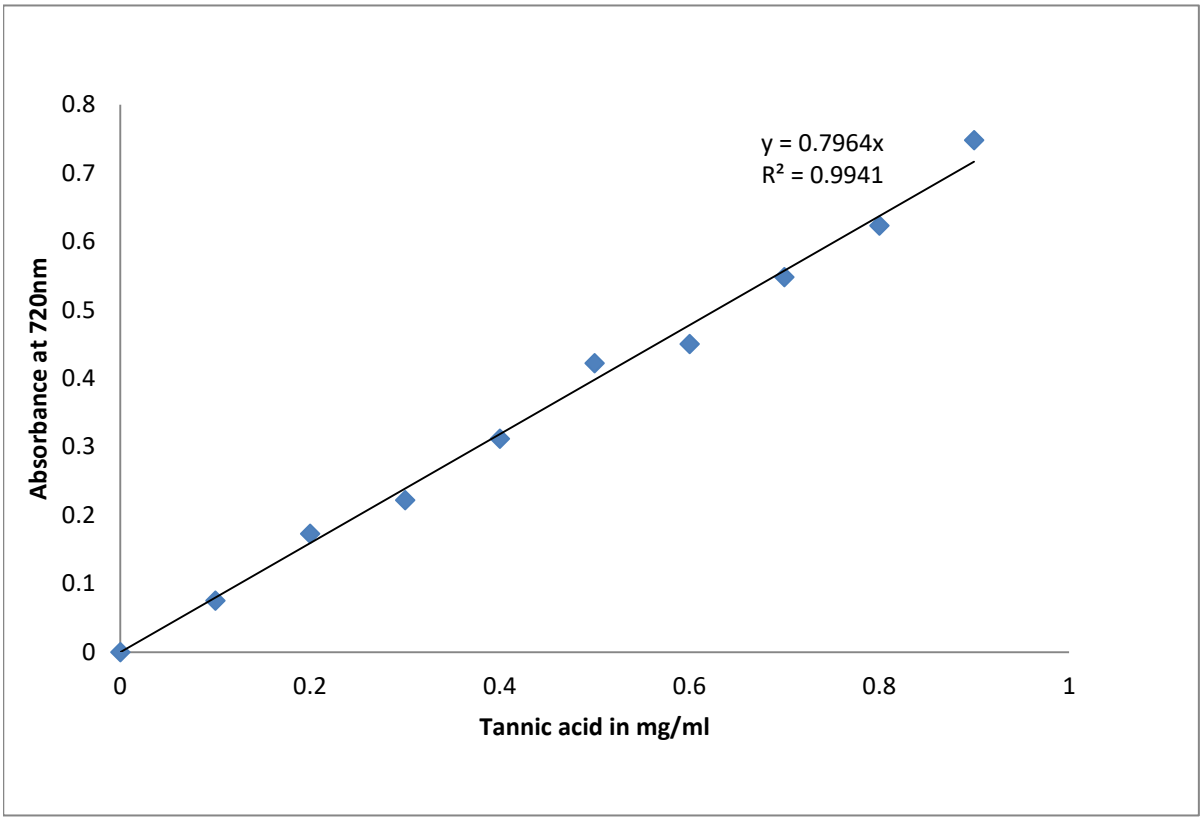
Appendix 1. Ranking of sampled beers based on the concentrations of the analyzed parameters

Beer samples	Total phenolics(mM)	Total tannin (mg/ml)	Anthocyanin (%)	Leucoanthocyanin (%)	Residual sugar (mM)
1	9th	12th	5th	8th	3rd
2	8th	7th	11th	6th	11th
3	3rd	4th	10th	9th	9th
4	4th	6th	7th	12th	5th
5	13th	8th	1st	1st	1st
6	7th	1st	9th	5th	6th
7	1st	9th	8th	4th	10th
8	11th	5th	2nd	2nd	7th
9	10th	11th	12th	11th	4th
10	6th	14	6th	10th	2nd
11	5th	10th	13th	1st	8th
12	12th	2nd	3rd	5th	3rd
13	14th	13th	4th	3rd	12th
14	2nd	3rd	14th	7th	13th

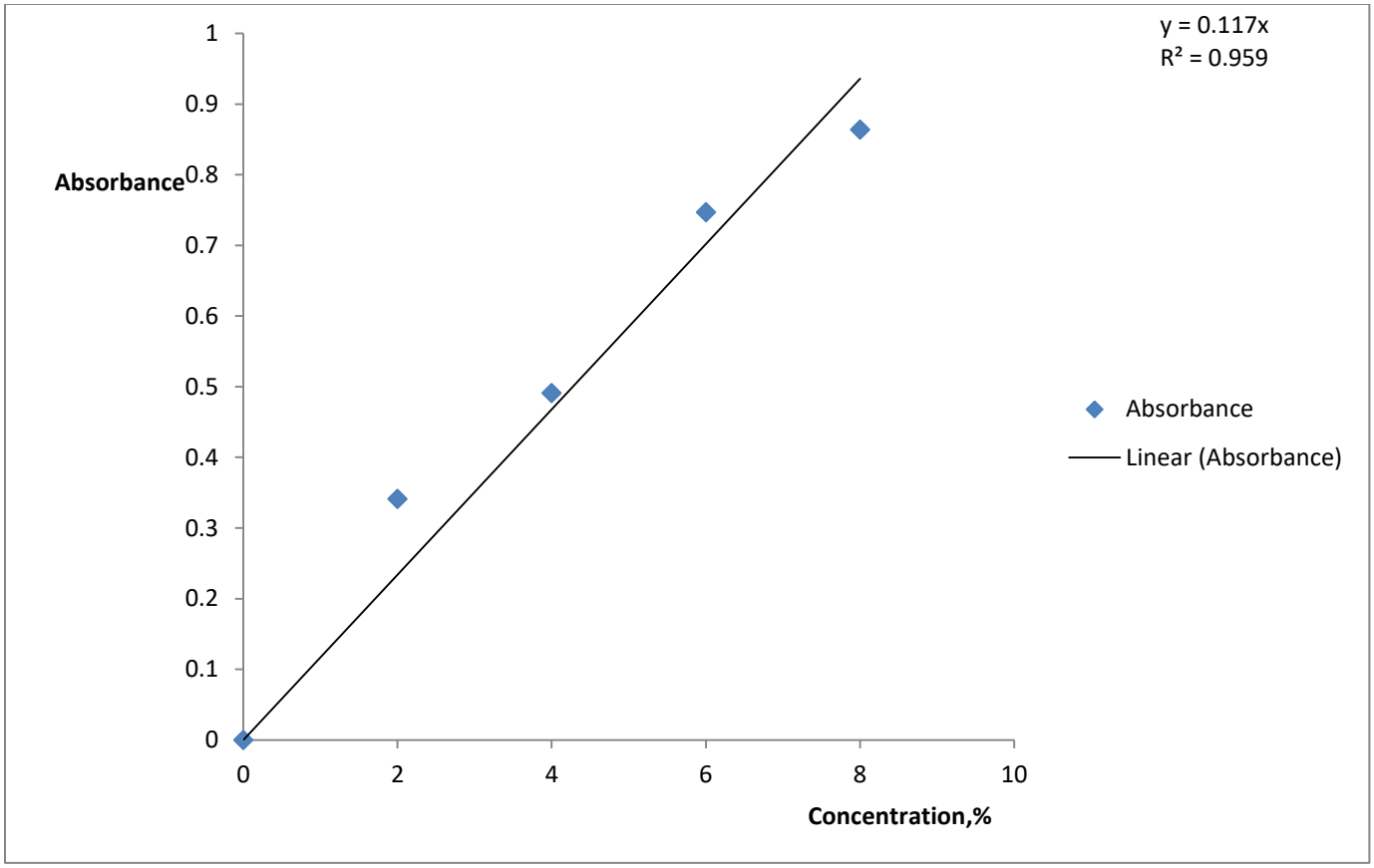
Appendix 2. Gallic acid calibration curve



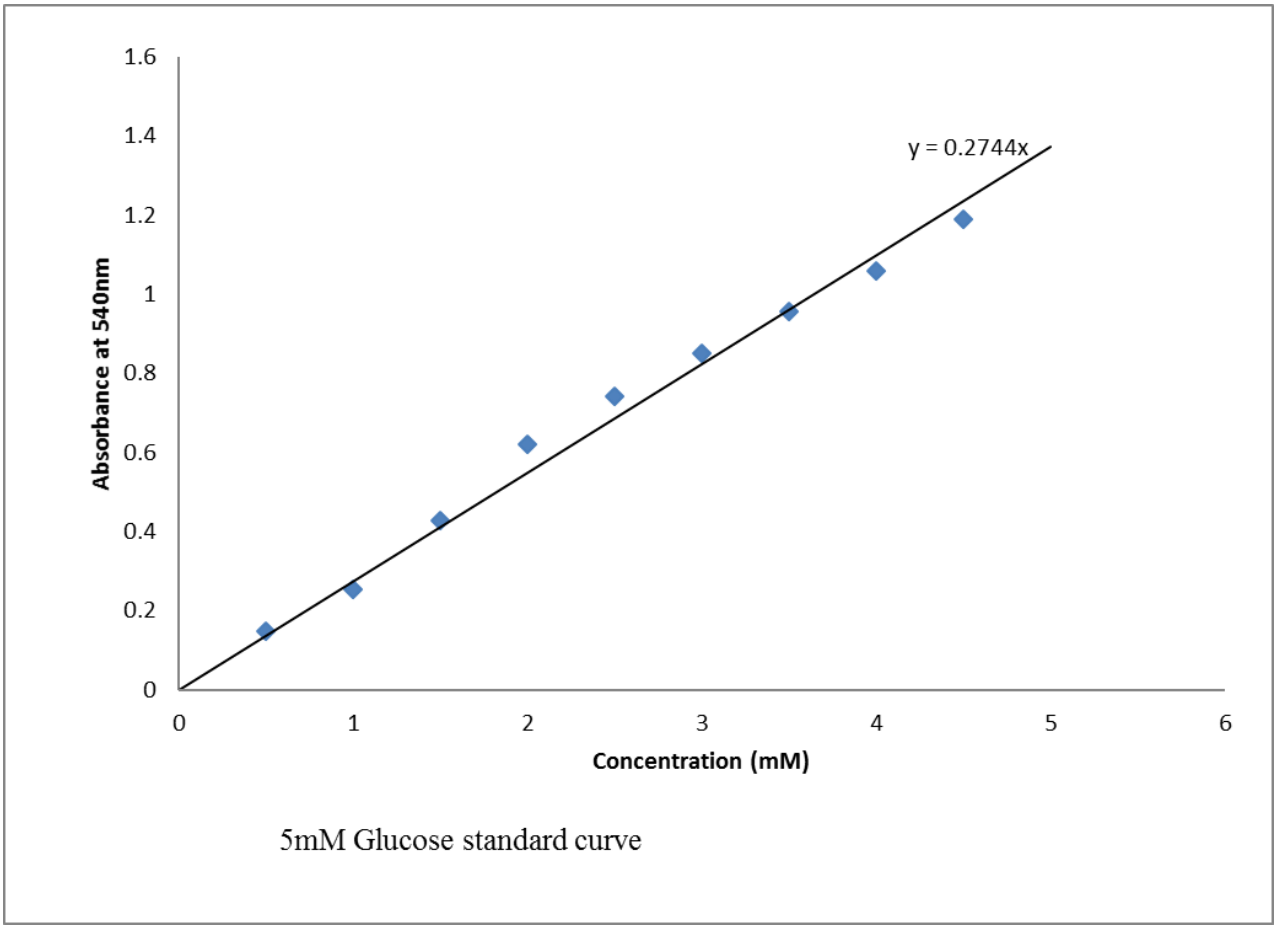
Appendix 3. Tannic acid calibration curve



Appendix 4. Cyanin calibration curve



Appendix 5. Glucose calibration curve



5mM Glucose standard curve

Appendix 6. Descriptive Statistics for total phenolics (mM)

	N	Minimum	Maximum	Mean	Std. Deviation
sample1	2	57.62	86.58	72.1000	20.47781
sample2	2	65.17	82.81	73.9900	12.47336
sample3	2	82.15	88.66	85.4050	4.60327
sample4	2	71.58	95.17	83.3750	16.68065
sample5	2	46.49	62.25	54.3700	11.14400
sample6	2	49.70	98.94	74.3200	34.81794
sample7	2	79.79	96.02	87.9050	11.47634
sample8	2	58.66	63.57	61.1150	3.47189
sample9	2	65.64	66.49	66.0650	.60104
sample10	2	64.79	91.32	78.0550	18.75954
sample11	2	79.42	86.87	83.1450	5.26795
sample12	2	57.81	62.62	60.2150	3.40118
sample13	2	30.17	41.02	35.5950	7.67211
sample14	2	85.64	90.08	87.8600	3.13955
Valid N (listwise)	2				

Appendix 7. Descriptive Statistics for Total Tannin Content of analyzed beer samples (mg/ml)

	N	Minimum	Maximum	Mean	Std. Deviation
sample1	2	1.22	1.37	1.2950	.10607
sample2	2	1.21	1.92	1.5650	.50205
sample3	2	1.74	1.87	1.8050	.09192
sample4	2	1.04	2.12	1.5800	.76368
sample5	2	.90	1.44	1.1700	.38184
sample6	2	1.94	2.09	2.0150	.10607
sample7	2	1.09	1.77	1.4300	.48083
sample8	2	1.54	1.69	1.6150	.10607
sample9	2	1.36	1.42	1.3900	.04243
sample10	2	1.05	1.29	1.1700	.16971
sample11	2	1.19	1.62	1.4050	.30406
sample12	2	1.89	1.95	1.9200	.04243
sample13	2	1.25	1.30	1.2750	.03536
sample14	2	1.48	2.22	1.8500	.52326
Valid N (listwise)	2				

Appendix 8. Descriptive Statistics for Total Anthocyanin (%)

	N	Minimum	Maximum	Mean	Std. Deviation
sample1	2	3.94	6.38	5.1600	1.72534
sample2	2	4.30	4.55	4.4250	.17678
sample3	2	4.36	4.62	4.4900	.18385
sample4	2	4.37	5.35	4.8600	.69296
sample5	2	5.15	6.27	5.7100	.79196
sample6	2	4.46	4.67	4.5650	.14849
sample7	2	4.30	5.02	4.6600	.50912
sample8	2	4.38	6.38	5.3800	1.41421
sample9	2	3.94	4.44	4.1900	.35355
sample10	2	4.28	6.02	5.1500	1.23037
sample11	2	3.76	4.46	4.1100	.49497
sample12	2	5.10	5.46	5.2800	.25456
sample13	2	4.79	5.64	5.2150	.60104
sample14	2	3.52	4.63	4.0750	.78489
Valid N (listwise)	2				

Appendix 9. Descriptive statistics for leucoanthocyanin (%)

	N	Minimum	Maximum	Mean	Std. Deviation
sample1	2	3.60	4.38	3.9900	.55154
sample2	2	3.85	4.34	4.0950	.34648
sample3	2	3.87	3.97	3.9200	.07071
sample4	2	3.50	3.97	3.7350	.33234
sample5	2	4.88	5.14	5.0100	.18385
sample6	2	4.41	4.74	4.5750	.23335
sample7	2	4.00	5.27	4.6350	.89803
sample8	2	4.46	5.12	4.7900	.46669
sample9	2	3.72	3.85	3.7850	.09192
sample10	2	3.57	4.20	3.8850	.44548
sample11	2	4.91	5.10	5.0050	.13435
sample12	2	4.25	4.91	4.5800	.46669
sample13	2	4.31	5.22	4.7650	.64347
sample14	2	3.51	4.68	4.0950	.82731
Valid N (listwise)	2				

Appendix 10. Descriptive statistics for residual sugar (mM)

	N	Minimum	Maximum	Mean	Std. Deviation
sample1	2	4.89	7.95	6.4200	2.16375
sample2	2	5.32	6.13	5.7250	.57276
sample3	2	5.86	7.00	6.4300	.80610
sample4	2	5.75	8.76	7.2550	2.12839
sample5	2	5.71	7.51	6.6100	1.27279
sample6	2	4.29	7.88	6.0850	2.53851
sample7	2	3.74	4.95	4.3450	.85560
sample8	2	5.49	6.86	6.1750	.96874
sample9	2	5.04	5.58	5.3100	.38184
sample10	2	7.44	7.71	7.5750	.19092
sample11	2	6.41	7.43	6.9200	.72125
sample12	2	6.28	8.42	7.3500	1.51321
sample13	2	4.44	6.97	5.7050	1.78898
sample14	2	1.99	3.43	2.7100	1.01823
Valid N (listwise)	2				