STABILITY OF PHENOLIC COMPOUNDS IN FREEZE DRIED BERRY PRODUCTS WITH TYPICALLY LONG SHELF LIFE

Master Thesis in Technology
University of Turku
Department of Life Technologies
Master`s programme in Food Development
1st of June, 2022
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UNIVERSITY OF TURKU
Department of Life Technologies
DIPANKAR BISWAS: Stability of phenolics compounds in freeze dried berry products with typically long shelf life
Master`s Thesis in Technology, 51 pages
Food Development
June, 2022

Berries are a potentially rich source of polyphenols, particularly anthocyanins, which may play an important role in the increased risk of chronic diseases. Anthocyanins, which are found primarily in fruits and vegetables, are gaining popularity due to their potential antioxidant activity, but they are highly susceptible to degradation during processing and storage. One of the most important quality characteristics of food products is the stability of anthocyanin content during processing and storage. Freeze-drying is a preservation method where water is removed by sublimation from the frozen material. Typically, the process is used with high-value raw materials, such as berries, which can be used as ingredients in mueslis and granolas.

Aim of this study was to evaluate the effect of storage on anthocyanin and flavonol compositions and contents and total antioxidant activity in different freeze-dried berries maintained at accelerated storage (40 °C for 1-6 months). Samples were muesli packages including freeze-dried strawberry, black currant or blueberry material prepared in Estonia. Identifications and quantifications of the phenolic compounds were carried out using HPLCDAD and HPLC-MS instruments.

Total anthocyanin found in Blackcurrant (at Day zero) 288.9 mg/100g dry matter, In Blueberry 836 mg/100g and in Strawberry 126.5 mg/100g. After two month of storage (56 days), all berries resulted in significant losses in anthocyanins with as much as 60% in strawberry, 60% in blueberry and 46% in blackcurrant compared to Day 0. In addition, after 56 days to 120 days' anthocyanin losses were less 21% in strawberry, 59% in Blueberry and 29% in Blackcurrant compared to Day 0 with 100%. In contrast, variety of flavonols detected and quantified with HPLC from the samples remained unchanged during the first months of storage.

Keywords

Blackcurrant, Strawberry, Blueberry, Anthocyanin, Identification, Degradation, Storage, HPLC-DAD

Contents

1. Introduction

The link between diet and health has increased consumer interest in learning more about nutraceutical-rich diets that include fruits and vegetables (Nöthlings et al., 2008 & Santos-Cervantes et al., 2007). Their bioactive constituents have positive effects on human health and help to prevent chronic diseases (Timlin & Pereira., 2007). Berries, which contain a wide range of phenolic compounds, may be included in healthy diets (Szajdek., & Borowska., 2008; Alwerdt et al., 2008); blackberry (*Rubus sp*.), bilberry (*Vaccinium myrtillus*), blackcurrant *(Ribes rugrum*), blueberry (*V. Corymbosum*), chokeberry (*Aronia melanocarpa*), cranberry (*V. Macrocarpon*), bayberry (*Myrica sp*.), raspberry (*R. Ideaus*) (*R. Occidentalis*) and strawberry (*Fragaria ananassa*) are commonly consumed in the human diet in both fresh and processed forms; interestingly, berries are exceptionally rich sources of antioxidant phenolics (Seeram et al., 2006 &Seeram., 2008). Flavonoids (anthocyanins, flavonols, flavones, flavanols, flavanones, and isoflavonoids), stilbenes, tannins, and phenolic acids are the most abundant phytochemicals in berry fruits (Seeram., 2008). Phenolics help to protect against degenerative diseases, and their health benefits have been attributed primarily to their antioxidant properties. Improving our understanding of their bioavailability is a critical step toward understanding the possible mechanisms of their health effects and characterizing the impact of berries on human health (Zhao., & Moghadasian., 2008; Lafay & Gil-Izquierdo., 2008).

One of the most critical quality aspects of food items is the constancy of antioxidant potential during processing and storage. Berries are a significant resource of polyphenols, especially proanthocyanins, which may help to lower the risk of degenerative disorders. Anthocyanins are gaining popularity in fruits and vegetables due to their prospective natural antioxidant, although they are easily degraded during manufacturing and preservation. Freeze-drying is a conservation procedure in which water is eliminated from freezing materials through sublimation. The method is typically utilized with high-value natural resources like berries that can be utilized in mueslis and granolas.

2. Literature Review

2.1 Background Information of berry fruits

Soft fruits and our health have long established associations steeped in tradition with strong linkage. Many traditional and folk medicines have been used berries from remedies for a wide range of health solution (Kellogg & Flint, 2010). Say for example north American indigenous people have used berries which is *Rubus* species for their health treatment like diarrhoea and for pain relief. However, there are so many evidences existed over the last twenty years showed that berries components have measurable beneficial effect on health (Seeram., 2012).

In botanic terms, Berries are defined as a freshly fruits that actually arises from entire plant ovary which mainly surrounds the seeds. Therefore, here true berries included bananas, grapes, blueberries, blackcurrants, strawberries and coffee beans as well. In this study, used those berries which are commonly uses now a day. This include soft berries with multiple seeds including strawberries, blueberries, blackcurrants etc.

2.2 Health Benefits of berries

Berries have two types of health benefits, which can be divided into nutritional and non-nutritional components. Berries are low in calories, fats, and sodium, but they do need essential minerals, dietary fiber, including soluble fibers like pectin, and vitamin C. **(Table:1**). Berries have a sweet taste due to the sugars they contain, such as glucose, fructose, and sucrose. Carotenoids, which are precursors to vitamin A, are found in all types of berries. As a result, the vitamin content of some berries, for example, is nutritionally significant. Black currants contain 40% of the RDA for vitamin K per 100g. Furthermore, strawberries can provide approximately 15-18% of the RDA for folate per 100g.

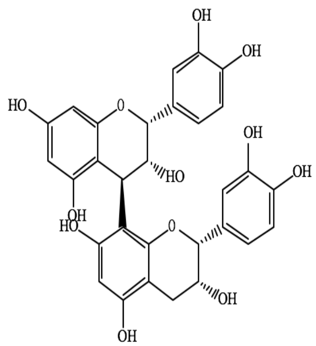
Berries are high in antioxidants, particularly polyphenols, carotenoids, and vitamin C. However, the amount of antioxidant components varies between species, varieties, and growing conditions. For example, berry species differ greatly in their vitamin C content, with black currants and strawberries having the highest levels (**Table 1**) and some berries, such as blueberries, having negligible levels (Walker., 2006). Black currant is high in carotenoids and fat, the majority of which is unsaturated. Total polyphenol content varies between berry species, varieties, and growing conditions. Blackcurrants and strawberries typically have total polyphenol contents of 300-100mg/100g (Hancock & Stewart., 2010).

Table 1: Nutritional components in selected berries (Extracted from the FINELI database)

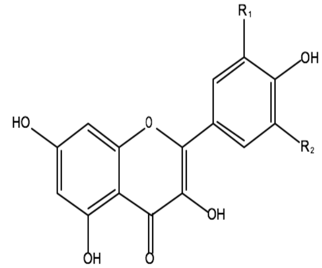
Components	Black currant	Strawberry	Blueberry
Macro-components			
Energy kJ (Kcal)	247 (59)	195 (47)	225 (144)
Carbohydrate, available (g)	7.8	8.4	6.4
Fat, total (g)	0.4	0.2	0.6
Protein, total	1.1	0.5	0.4
Carbohydrate components (g)			
Organic acids, total	2.7	1.6	1.4
Starch, total	0	0	0
Sugars, total	7.8	8.4	6.4
Sucrose	0.3	2.3	0.5
Glucose	3.5	3.1	3.0
Fructose	4.0	3.0	2.9
Fibre, total	5.8	1.9	3.3
Fibre, water-insoluble	3.0	1.5	2,6
Non-cellulosic polysaccharides, water-soluble	1.9	0.9	0.5
Fats			
Fatty acids, total (g)	0.1	0.2	0.3
Fatty acids, total saturated (g)	< 0.1	< 0.1	< 0.1
Fatty acids, monounsaturated* (g)	< 0.1	< 0.1	< 0.1
Fatty acids, polyunsaturated (g)	< 0.1	0.1	0.3
Linoleic acid (mg)	45	64	125
✔-linolenic acid (mg)	27	64	143
Cholesterol (mg)	0	0	0
Total sterols (mg)	8.8	10	17.8
Minerals (mg)			
Sodium	0.5	0.7	0.7
Salt	1.3	1.8	1.8
Potassium	340	190	190
Magnesium	24	15	15
Calcium	72	21	21
Phosphorus	58	30	30
Iron	1.2	0.5	0.5
Zinc	0.3	0.1	0.1
Iodide (g)	1.0	1.0	1.0

Selenium (g)	0.1	0.1	0.1
Vitamins and others			
Vitamin A [retinol activity equiv. (g)]	8.2	0.9	0.9
Vitamin D (g)	0	0	0
Vitamin E [tocopherol (mg)]	2.2	0.6	0.6
Vitamin K (g)	30	5.5	5.5
Vitamin C (ascorbic acid, mg)	120	60	60
Folate (g)	7.7	35.6	35.6
Niacin equivalents (g)	0.5	0.7	0.7
Riboflavin	0.07	0.07	0.07
Thiamin (vitamin B1, mg)	0.05	0.03	0.03
Pyridoxine vitamers (mg)	0.08	0.06	0.06
Carotenoids (g)	542.2	44.5	44.5

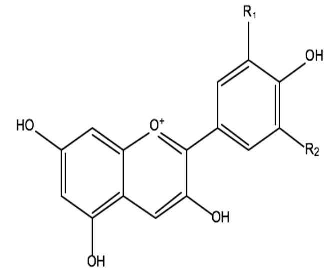
The composition of polyphenols can be defining the color and palatability of different berry species and influence their possible beneficial effects on health. Polyphenols are diverse family of components which differ in structure and potential bioactivity. The different color of berries like red to purple to blue is due to the presence of polyphenols pigments called anthocyanin. **(Figure: 1).**



The proanthocyanidin dimer B2. Proanthocyanidins are found in a range of berries and are associated with a range of health benefits.

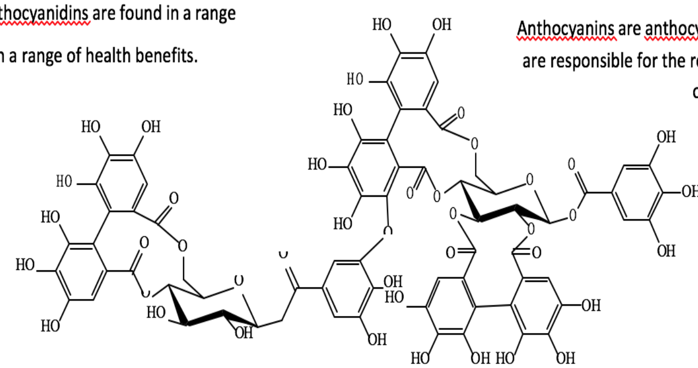


Flavonol	R1	R2
Kaempferol	H	H
Quercetin	OH	H
Isorhamnetin	OCH_3	H
Myricetin	OH	OH



Anthocyanidin	R1	R2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OH	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Anthocyanins are anthocyanidins with attached sugars and are responsible for the red-to-blue-to-purple colouration of berries



The ellagitannin, Sanguiin H-10, is one of the main antioxidant components in raspberry and contributes to its characteristic astringency.

Figure 1: Structures of polyphenol in Berries

Their composition varies between berry species. The red-orange color of strawberries is caused by the presence of pelargronidin type anthocyanins, whereas the deep purple-black color of black currants is caused by an accumulation of delphinidin and cyaniding type anthocyanins in the skin. Berry flavors, on the other hand, are driven by acid sugar balance and polyphenol components. Strawberry contains tannins such as ellagitannins and proanthocyanins, which contribute to the astringency of these berries (de Freitas & Mateus., 2012).

Over the last two decades, there has been a growing consensus that polyphenol components' high antioxidant capacity may contribute to health benefits by mitigating the negative effects of reactive oxygen species (ROS) generated in the body through oxygen metabolism.

Polyphenols were proposed to act as chain-breaking agents, preventing ROS from initiating free radical cascades that could damage cells, DNA, and membranes and cause diseases (Halliwell, B. 2007). This straightforward and appealing principle, however, is not universally accepted, and high antioxidant capacity in the test tube does not always translate into in vivo effectiveness. Although berry polyphenols have a high antioxidant capacity in the test tube, their efficacy is limited by their low blood uptake. Even a small amount taken up in the serum is weakened by further metabolism in the liver and excretion via the bile or urine (Koli et al., 2010). In many cases, the original components are effectively absent, and their circulating metabolites may differ significantly in structure and potential function (Williamson & Clifford.,2010). It should be reiterated that different polyphenols have varying stabilities, bioavailability, and thus potential efficacy.

2.3 Chemical Composition of Berries

Berries' chemical makeup varies based on the cultivar, species, and variation, as well as the place where they are grown and environmental factors. It also depends on the stage of ripeness, the time of harvest, and the following storage conditions. As a result, the quantity of every single element, as well as the quality of the fruits, varies greatly. Berries are often high in vitamins, including ascorbic and folic acid, as well as phytochemicals such as polyphenols. As a result, these compounds could be a great alternative for nutrition and other industries looking to use functional food products. Berries also contain high contents and wide diversity of nonessential biologically active components such as organic acids or polyphenols, with their subclasses, tannins and flavonoids (Giampieri et al., 2015 Joseph et al., 2014; Jimenez-Garcia at al. 2013). Anthocyanin a subclass of flavonoids are important bioactive compounds in berries. Bioactive compounds play an important role to provide and active cellular antioxidant protection, inhibit inflammatory gene expression. There bioactive compounds have been associated with protective effects against chronic diseases like cancer, cardiovascular diseases (CVD), Alzheimer’s, Depression and others (Joseph et al. 2014 & Khalid et al. 2017).

Strawberry and blueberry are two of the most demanded berries in fresh and frozen forms. So there numerous in vitro and in vivo studies shown that strawberry and blueberry reducing the risk of several chronic diseases (Forbes-Hernandez et al. 2016; Nile et al. 2014). The chemical composition of strawberry and blueberry are compared in (**Table 2**).

Table 2: Chemical composition of strawberry and blueberry (Rothwell, J. A. et al. 2013)

	Strawberry	Blueberry
Flavonoids		
Anthocyanins (mg/kg FW)	73.0	134
Flavonols (mg/kg FW)	9.1	1.1
Phenolics acids		
Hydroxybenzoic acids (mg/kg FW)	5.7	1.5
Hydroxycinnamic acids (mg/kg FW)	7.1	135.0

2.4 Phenolics compound

Is made up of phenolic chemicals. Phenolic chemicals are a collection of tiny molecules present in most plant parts, especially fruit and veggies, that include at minimum one polyphenol element in its sequence. Polyphenolic compounds, flavonoids, polyphenols, coumarins, lignans, phenolics, glycosides, and polyphenol are some of the subgroups of phenolic composites that may be separated influenced by the chemical composition. The graphic below depicts the many types of polyphenols, and they're complementary. In the land plants, natural antioxidants are accessible in a dissolved form if ordered by the pattern in which they originate. Plants' internal plasma membrane and vacuoles produce the majority of soluble polyphenol chemicals. Nevertheless, total phenolic complexes are synthesized when soluble polyphenols are transported to the cell wall, where they are subsequently connected with cell wall components, including cellulose and polypeptide via ester and glycosidic linkages, adding to cell wall synthesis.

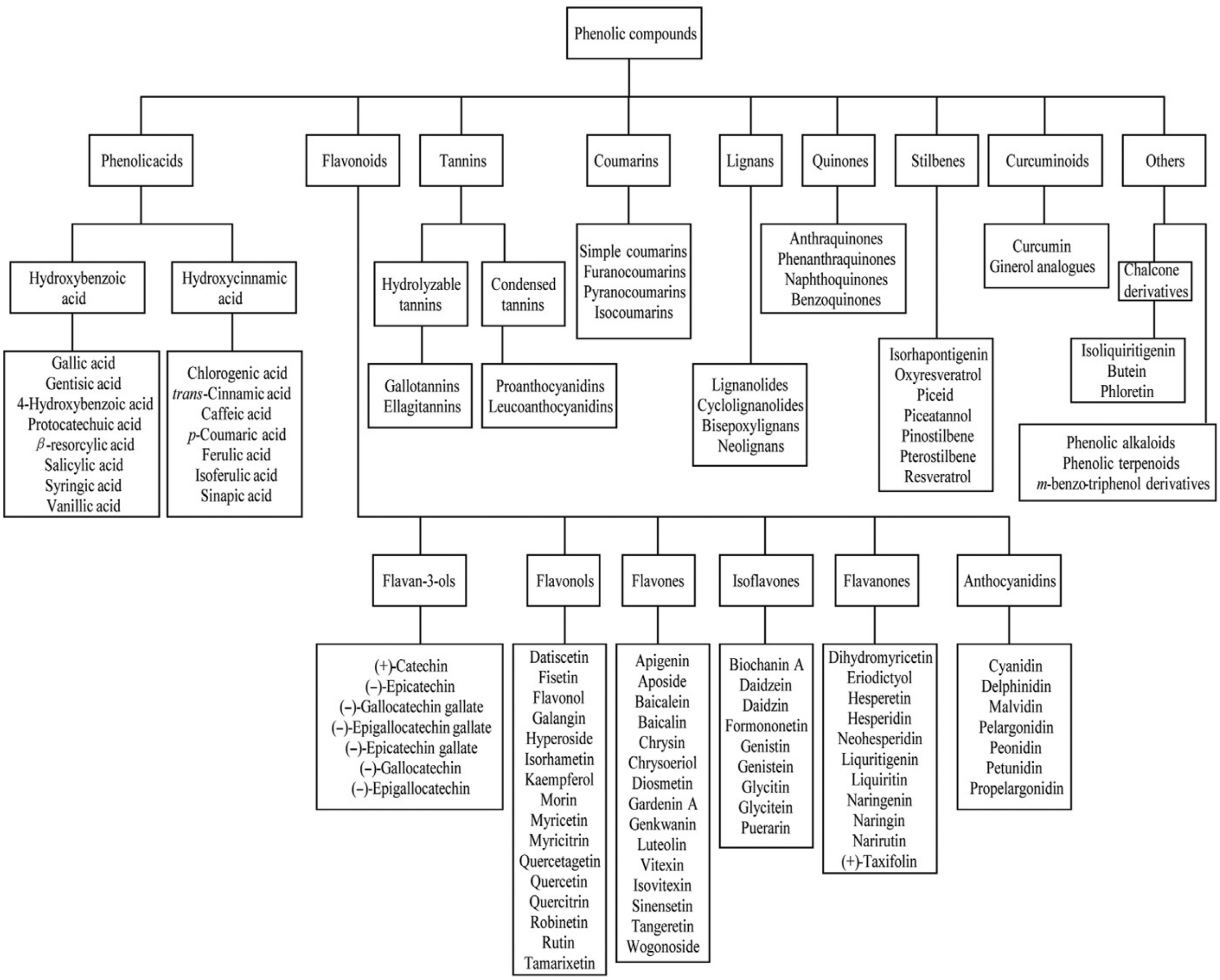


Figure 2: Categories of phenolic compounds

2.5 Berries' phenolic composition

Anthocyanins are polyphenolic chemicals found in berries that are implemented widely in fruit color and employed as natural sources of color, for example, red and blue hues in the food business. Berries have higher anthocyanin levels than other foods. Their qualities are influenced by the climate, cultivar choices, light, heat, and agricultural practice. Anthocyanins degrade at different rates depending on time and temperature. Ph is another element that influences color and anthocyanin structure. Reaction rates in phenolic substances, such as anthraquinones can be influenced by pH changes. Because total anthocyanin concentration is connected with antioxidant capacity, lower pH (2.5) is better for the conservation of polyphenol in berry products throughout preservation for a few weeks than better amounts.

2.6 Berry flavonoids, tannins, and phenolic acids

According to Mäkilä's paper, some of the most common phenolic groups found in berries are flavonoids, tannins, and phenolic acids. These chemicals, which are mostly secondary metabolites, are found in higher plants. A lot of them function as plant pathogen and herbivore defense mechanisms. The chemicals are often caused by numerous stressors, particularly UV light (El-Seedi et al. 2012). They also serve as cell membrane elements (Wallace & Fry 1994). It contains a variety of colorful attractants for birds and insects, particularly for pollination and seed dispersion (Andersen & Markham 2005). These chemicals are often found in plant tissues as simple substitution phenols, mostly as glycosides or as complex polymers.

Berry fruits are rich in phenolic compounds such as phenolic acids, flavonoids, lignans and tannins (**Figure 3**). Most flavonoids, anthocyanidins are found in nature as glycoside which is made of sugar and another molecule called anthocyanin. These anthocyanins can be absorbed in their whole form that is linked to different sugar.

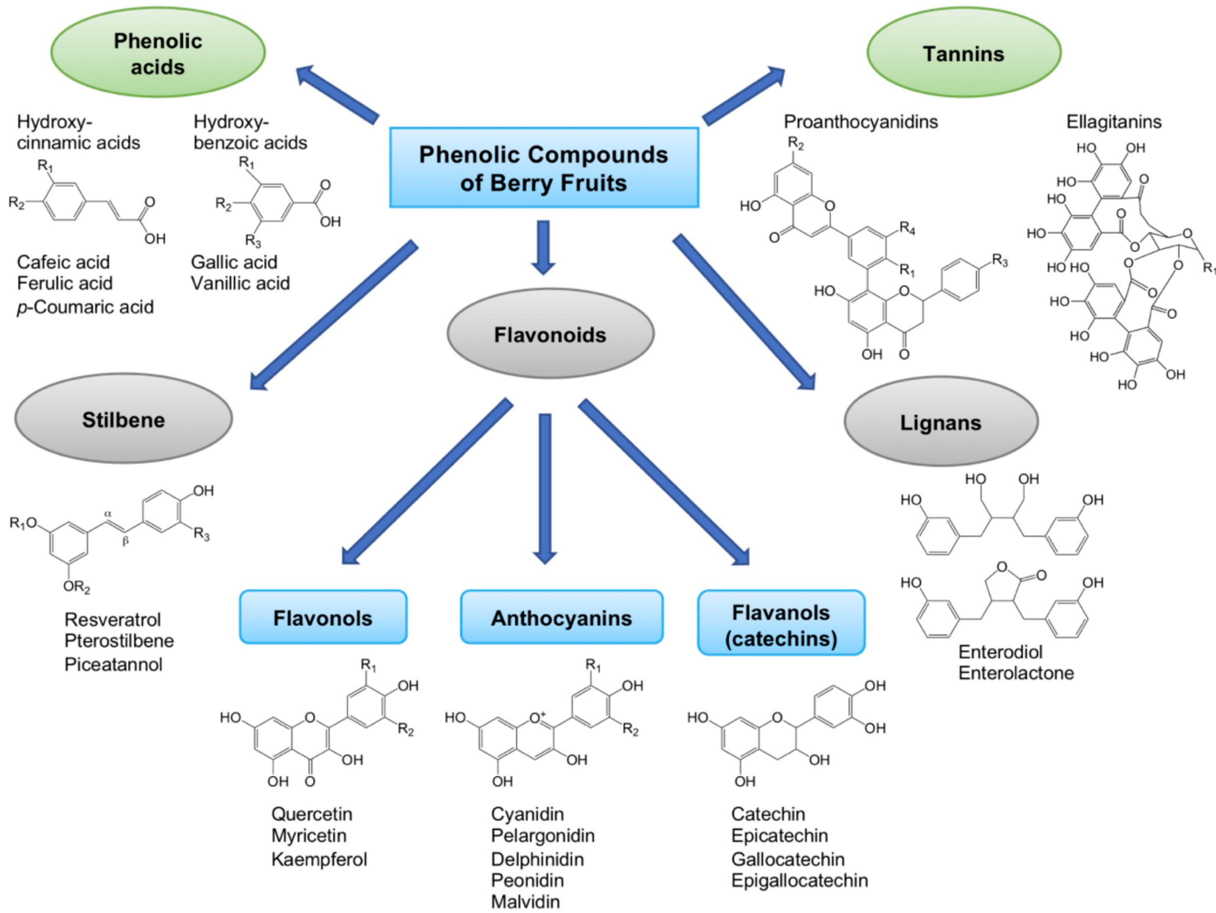


Figure 3: Classification and chemical structure of phenolic compounds in berries. Adapted from Paredes-López et al., 2010 and Nile & Park. 2014

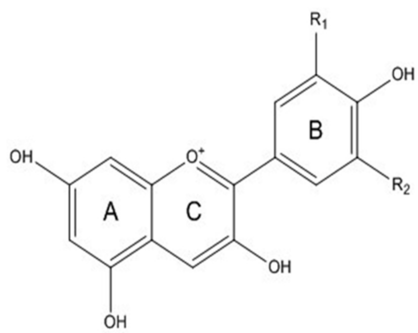
Flavonoids, together with anthocyanins, flavonols, and flavan-3-ols, have the C6-C3-C6 polyphenolic nucleus, which is made up of two benzene rings (A- and B-rings) joined by an oxygen-containing found a positive association or pyrone ring (C-ring). Natural antioxidants are the most abundant flavonoids in berries, and they are responsible for their dark hue (Määttä et al. 2001). Anthocyanins are water-soluble pigments that give berries their blue, violet, and magenta hues (Clifford 2000). Greater than twenty empirical results confirm they have been discovered, although delphinidin, cyanidin, peonidin, pelargonidin, petunidin, and malvidin are the most significant (Kula et al. 2016)

The polyhydroxylated substituent cationic is the structural and functional unit. In nature, the hydroxyl group at C-3 is invariably glycosylated by 3-O-glycosides such as glucose, rhamnose, or rutinose, and further glycosylation can occur on hydroxyl groups at C-5, C-7, C-3', C-4', and/or C-5'. Furthermore, several aliphatic or aromatic acids can acylate the sugar units (Iacobucci & Sweeny 1983). Flavonols are light yellow chemicals that are found in raspberries as O-glycosides. A dual bond connects C-2 and C-3, a carbonyl group connects C-4, and a hydroxyl group connects C-3. C-3 (3-O-glycosides) is the predominant location for glycosylation, with C-7 being used less commonly. Furthermore, the sugar component in flavonols may be acylated (Andersen & Markham 2005). A hydroxyl group is also present in flavanols (flavan-3-ols). Flavonoids have quite a hydroxyl group at C-3 but no straight bond connecting C-2 and C-3, and no aromatic ring at C-4. At C-2 and C-3, they have chiral centers (Beecher 2003; Treutter et al. 1994). Polymeric concentrated tannin is made up of flavan-3-ols, which are the structural components.

Greenhouse gas linkages across C-4 and C-8 of the neighboring monomer link the flavanol monomers straight or branching when connected across C-4 and C-6. These monomer connections result in polymers with 2 to 50 units (or more). Proanthocyanins are another name for condensed tannins. The molecules are not subject to hydrolysis cleavage (Wheeler 1979). Under acidic circumstances, proanthocyanins can be transformed to consist of a large number by high heat treatments, especially for low moisture situations (Porter et al. 1985). Hydrolyzable tannins include targeted treatment and polyphenolic compounds. A carbohydrate, generally glucose, is in the heart of a hydrolyzable tannin monomer, and its OH-groups are partly or completely esterified with phenolic acids. The phenolic acid in ellagitannins is ellagic acid, while gallic acid is found in gallotannins. Amino electrolytes or weak bases solubilize these substances to generate carbohydrate and polyphenols acids.

2.7 Chemistry of Anthocyanin

Anthocyanin belongs to a large group of compounds collectively known as flavonoids which are subgroup of an even larger group of compounds known as polyphenols (Mcghie & Walton., 2007). Chemically anthocyanins occur as glycosides of flavylium (2-phenylbenzopyrylium) salts but differ from them by structural variations in the number of hydroxyl group. At the point of methylation, these hydroxyl groups, the number and nature of sugar moieties attached to the molecule and the position of the attachment, as well as the nature and the number of aliphatic and aromatic acids attached to the sugars (Ramawat et al. 2013). The anthocyanidins consists of an aromatic ring A bound to the heterocyclic ring C which contain oxygen, that is also bound by a carbon-carbon bond to a third aromatic ring B (Ignat et al. 2011). There are nearly 17 anthocyanidins have been found but only six of them are commonly distributed in the nature, cyaniding(cy), delphinidin (Dp), malvidin (Mv), pelargronidin (Pg), peonidin (Pn) and petunidin (Pt) (Figure 4).



Anthocyanidin	R_1	R_2
Cyanidin (Cy)	OH	H
Delphinidin (Dp)	OH	OH
Malvidin (Mv)	OCH_3	OCH_3
Pelargonidin (Pg)	H	H
Peonidin (Pn)	OCH_3	H
Petunidin (Pt)	OH	OCH_3

Figure 4: Anthocyanidins structure

Anthocyanin are highly instable and susceptible to degradation. There is some factor which affect the chemistry of anthocyanins also their stability and color as well (Fernandes et al. 2014). The hue of anthocyanin might be varying according to different substituent groups that present on the B ring, and color saturation increase with increasing number hydroxyl groups and decreases with the addition of methoxyl groups (Tsuda, 2012).

In aqueous solution, anthocyanins undergo structural re-arrangements in reponse to change in pH in four molecular structure which are quinoidal base (blue), flavylium cation (red), carbinol (Colorless) and chalcone (yellowish) forms **(Figure 5**). Anthocyanins are stable in acidic condition pH between 1 to 3 where they exist primarily as flavylium cations. But at pH>4 **anthe cathocyanins** adopt the forms of the carbinol and chalcone. After that chalcone undergo chemical degradation and to produce phenolic acids (Fang, 2014). The different molecular structure of anthocyanins coexisting in aqueous solution at any given time will depend on pH, temperature and time.

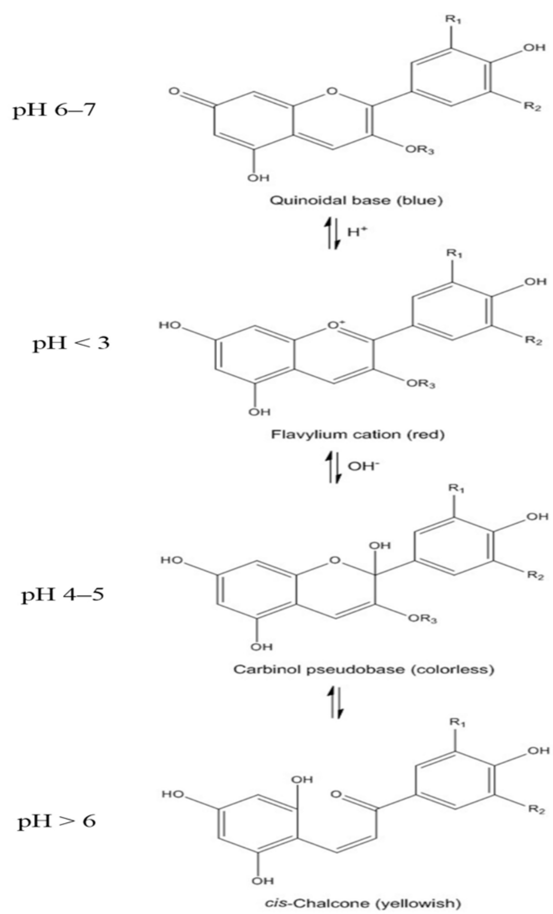


Figure 5: Molecular structure of anthocyanin under different pH condition.

3. Effect of preservation on phenolics in berries

Since many types of fruits are seasonal and their shelf life is limited for time being so they must be processed to keep the quality (Ścibisz & Mitek 2007). Processing may include preservation by different method such as adding of sugar to make a jam, fermentation and drying

Storage causes phenolic compounds to decompose in a similar way as heat processing, although the endpoints are strongly reliant on temperature range, light, oxygen, oxidizing native enzymes type, and cultivar. Reduced storage temperature, light-shielding, pectins, and high carbohydrate content in the goods have all been proven to be beneficial for color preservation.

The study of the isolation and identification of active phenolic systems in different plant-derived diets has sparked enthusiasm in the role of antioxidant compounds in public health (Shahidi & Naczk, 1995). Plants generate a wide range of phytonutrients, many of which are high in antioxidant acid removal and are widely investigated for their imperative results on human and animal's health under certain circumstances. The majority of these phenolic chemicals are found in human food and are also used in therapeutic treatments (Shahidi & Naczk 1995). Polyphenols and nutrients such as important Vitamins have been demonstrated in several scientific research to be significant elements in fruit and veggies that make significant contributions to health.

The berry fruits have a high-water content as well as sugar and require specific post-harvest issues that cause enzymes to lose momentum biochemical pathways and microbial to develop, leading to longer shelf life and, in general, products with longer life. Owing to the high levels of spoiling capability and seasonal, berry fruits are frequently treated or exposed to composite manufacturing that removes enormous volumes of water, delaying and interrupting unwanted processes that contribute to the destruction of the product's nutritional content. A freezer is categorized as the most efficient technique for preserving and storing fruits for long periods of time with minimum influence on nutritional content. In general, decreasing the temperatures of raw resources below the freezing point inhibits several metabolic activities in the fruits, slows microbial growth dynamics, and eventually avoids the breakdown of essential phytochemicals.

The main major limitation of the cooling process is a decrease in the level of the water as a necessary consequence of water involvement by crystal growth in the form of ice crystalline, resulting in reduced merchandise freshness, conformational changes of amorphous soluble compounds, which can decrease the quality of some essential bioactive components and primarily affect the displacements of the composition and framework of the product, as well as deterioration of the product's particular color. This is greatly influenced by the size and location of the ice crystals generated, which, relying on the type of freezing method used, may result in more or lesser damage to biological membranes and morphological structure deterioration, resulting in poorer product quality after thawing. On the contrary extreme, some writers claim that specific cooling procedures have a beneficial impact on the natural object's operational qualities since freezing can promote the release of bound molecules such as polyphenolic compounds and proanthocyanidins bioactive substances.

As a result of this, different cryoprotection technologies are being developed, i.e., the use of considerably lower subzero weather (cryogenic), which allows the feedstock to reach its melting point in a brief period, as little as a few seconds. Quick-freezing techniques can last anywhere from a few moments to a few minutes, dependent on the coolant (supercooled gas) utilized, for example, utilizing liquid nitrogen at temperatures as low as 196°F. In reality, quick-freezing procedures allow for the production and development of tiny crystallites in higher numbers, which do not cause as much harm to the plant cell and, as a result, to other physiochemical aspects of the product as traditional freezing methods (at 18°C for 24 hours).

HPLC has been shown to be the most efficient method for detaching and categorizing phenolic secondary metabolites in plant materials when combined with diode array detection for repeated analysis and spectroscopy for an elaborated approach, particularly for the polyphenolic identifier. The provisional attribution and classification of phenolic compounds into correlating groups is enabled by their UV vis spectroscopic analysis. Still, the characterization of the many various forms of phenolic compounds can be accomplished by incorporating these data with mass spectrometric data and analysis from the literature. Electroporation is a popular ionization technique for determining molecular weights and mass spectroscopy for determining phenolic compound structural characteristics (Gavrilova et al,.2011).

The amount and content of anthocyanins in berry species are influenced by several internal and external variables. These variables include genetic diversity and a variety of environmental conditions, including light intensity, humidity, temperature, fertilizer and pesticide usage, wounds and infections, and other stressors (Kahkonen et al., 2003). Recent harvested berries fruits are only accessible during specific days on a monthly or weekly basis of the year due to weather circumstances. As a result, several processing procedures are used to retain their strong flavor and freshness even when they are not in season. Berries are used to make juice, alcohol, jam, and food colorants, among other things. Anthocyanins arise in strawberries and other plants during the start of ripening when the activity of specific enzymes in the phenylpropanoid pathways increases, and they continue to accumulate during maturity (Slatnar et al., 2012). Although the development of anthocyanins coincides with the buildup of carbohydrates, no causal link has been identified. Food acceptance is heavily influenced by color. Colorants have been used in the food business for generations to improve or return the initial look of meals or to guarantee uniformity, and synthetic dyes have been used as an indication of food quality for millennia (Giusti & Wrolstad, 2003).

Jam production is one of the most common and widely used fruit preservatives, and it may not only extend the time they take on the shop or grocery of fruits but also enhance their accessibility before next (Al-u'daft et al., 2011). Jams are fluids mucilaginous food items made by heating a mixture of one or more fruits, glucose, and liquid to obtain a soluble solids percentage of more than 68 percent (Carle, & Schieber, 2005). In jam recipes, various ingredients such as pectin or phenolic compounds such as citric acid or acetic are commonly employed. From the moment it is made until it is eaten, the quality of jam begins to deteriorate (Ferreira et al., 2004). Processing and strongly recommended temperatures are some of the elements that have been hypothesized to affect the jam shelf life of the products. (Touati et al., 2014), for example, briefly demonstrated that over 60-day prolonged storage, the association time had a substantial influence on ph, total sugar and soluble protein concentration, and the sensory profile of apricot, which is namely Prunus armeniaca jam.

Other additions used in jam compositions include pectin and organic acids such as citric or acetic acid. From the moment it is produced until it is consumed, the quality of jam begins to deteriorate (Ferreira et al., 2004). Production and storage duration and temperature are some of the earlier research that revealed that jam quality and shelf life are influenced by a variety of parameters. For example, during a sixty-day storage time, according to Touati et al. (2014) found that the mixture time temperature had a substantial influence on acidity level, total sugar and soluble protein content, and the sensory profile of peach jam.

Fresh berries that are high in bioactive components are perishable. Elimination of water is one of the most efficient ways to increase the shelf life of berry products while preserving the vitamins and active ingredients of the berry to a large extent. Nevertheless, drying processes have a significant impact on the depth of flavor of the finished product. As a result, the current study covers the research status of early drying, use of freezers, suction drying, microwave drying, and revolutionary drying technologies for strawberry drying from the viewpoints of classic drying techniques and creative drying ideas. Various berries' dehydration mechanisms, pretreatment methods, and drying technologies were summarized. Furthermore, the impacts of various drying therapies on the nutrient content of berry drying were presented, and the implementation status of applied mathematics used in berry drying. Furthermore, the future research guidance and development trend of berry drying techniques were identified, which are directed at enhancing the drying rate of berries, conserving the active ingredients of berries to the maximum extent possible, and supporting the handling usage of strawberry and financial advantages in the future to continue providing a reference for further drying space technology and consumption.

Polyphenols, which are antioxidants found in fruits and vegetables, have become a study focus in recent years. Their critical benefits to human health include reducing tumorigenesis and slowing the onset of neurological and cardiovascular illnesses. Moreover, polyphenol has the ability to regulate the decay process of cyclooxygenase, lipoxygenase, and intracellular receptors (Sadik,2003). Berries-related fruits, small fruits, or berry typically refer to any little fruit that lacks seeds and may be consumed whole and are advocated for eating a diet due to its high bioactive nutritional content. Such fruits are commonly available and include blackberry blueberry, which is also known as *Vaccinium corymbosum*, red raspberry, also known as *Rubus idaeus*, and strawberry (*Fragaria Sativa*). As the bulk of fruits, Berries are known to be high in polyphenols, particularly anthocyanins (Pascual-Teresa, 2010). Previous research has focused on anthocyanins as cardio- and neuro-protective substances that prevent cancer proliferation and tumor growth.

Furthermore, polyphenols improve insulin sensitivity in adipocytes (Tsuda, 2006). Anthocyanins also demonstrated promising "in vitro" effects on glucose metabolism, inhibiting amylase and glucosidase. Notwithstanding their biological activity, the bioavailability of polyphenols in general, notable anthocyanins, is extremely low, ranging from an approximation of two to seven percent (Manach, 2004). Knowing the digestion and absorption of phenolics might thus contribute to a broader knowledge of the biological attributes. "In vitro" investigations are a quick, easy, and safe way to replicate the intestinal tract.

The composite juice had the lowest phenolic component level and most labile composition, resulting in the lowest color stability of the juice over lengthy storage. Due to its high phenolic concentration, EPR-juice is likely too astringent for customers, and this juice might be used as a supplementary component to increase the polyphenol content of fruit and strawberry products. The concentration of anthraquinones and phenolic acids varied the most extraordinary following thermal processing and storage at room temperature among the 51 metabolites tested. To maintain the original quality of any of the berry juices, cold storage is required.

3.1 Anthocyanins

Anthocyanins are a type of flavonoid that is made by plants and can be found in a wide range of secondary metabolites. Anthocyanins are glycosylated polyphenolic substances that may range in hue from orange to purple to blue. These molecules can be found in flowers, seeds, fruits, and the vegetative tissues of plants (Tanaka, 2008). The hue, or color attribute, of anthocyanins is affected by the intravacuolar environment. This is due to the fact that anthocyanins are water-soluble pigments that are predominantly located in cell vacuoles. There have been approximately 600 different anthocyanins identified in nature (Smeriglio et al., 2016). The anthocyanins that are found in plants the most often are pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin. These six anthocyanidins are widely scattered (Kong et al., 2003). Anthocyanins, which are known for their strong antioxidant capabilities, provide defense for plants against a wide variety of threats, both biotic and abiotic (Chalker-Scott, 1999; Ahmed et al., 2014). Another reason for the rise in popularity of foods high in anthocyanins is that these pigments give foods their vibrant colors and may provide some potential health benefits (Pojer et al., 2013).

3.1.1 Down-regulation of Anthocyanin Biosynthesis

The rates of production and degradation are what define the amounts of anthocyanin. Because of a shift toward degradation that is driven by anthocyanin biosynthetic downregulation, the amount of anthocyanin in the plant gradually diminishes until it is eliminated entirely. Tomatoes, eggplants, and peppers that have not yet reached maturity often develop anthocyanins in their skins, which gradually disappear as the fruit matures (Povero et al., 2011; Mennella et al., 2012). Anthocyanin discoloration in purple pepper fruits was associated with decreased expression of positive regulatory genes such as camyba and the majority of its downstream structural genes, resulting in decreased anthocyanin synthesis relative to degradation. This was determined by measuring the ratio of anthocyanin production to degradation (Borovsky et al., 2004). Anthocyanins accumulated at all stages of ripening as a consequence of the constitutively overexpressed positive transcription factors of the anthocyanin pathway, as shown in the transgenic Del/Ros1 tomato. This resulted in a fruit that had a dark purple color when it was fully ripe (Maligeppagol et al., 2013). Kiferle et al. (2015) used the constitutive camv 35S promoter to overexpress two tomato MYB genes that are similar to one another. These genes are slant1 and slan2. In all of these cases, this led to a large amount of anthocyanin coloring being produced in the immature fruits. This high color was only maintained in the mature 35S: ANT1 fruits, but the anthocyanins were mostly eliminated in the mature 35S: slan 2 fruits as they ripened. As a consequence of this, a decrease in the expression of anthocyanin activator is essential for a reduction in the creation of anthocyanin. Even when anthocyanin activators are produced in a constitutive manner, the ultimate anthocyanin content of ripe fruits may be controlled by which regulatory genes are expressed and their ability to activate downstream structural genes. This may be the case even though anthocyanin activators are produced.

3.1.2 Anthocyanin Pathway Environmental Regulation

There is some evidence that environmental factors may affect anthocyanin metabolism. Low temperature (Lightbourn et al., 2007), high irradiance (Lightbourn et al., 2016), UV/blue Light (Guo and Wang, 2010; Jiang et al., 2016), and low temperature (Lightbourn et al., 2007) all increased anthocyanin production, whereas high temperature caused its breakdown (Qiu et al., 2016; Movahed et al., 2016).

3.1.2.1 Temperature

When it comes to extending the shelf life of food and maintaining its quality, one of the most common and widely used techniques is heat processing. The specified shelf life of the manufacturer and the functional features of the food both play a role in determining the optimal temperature range for the heat treatment process, which may range anywhere from 50 to 180 degrees Celsius. Because food is cooked at high temperatures for extended periods of time, the color of the food, the number of anthocyanins it contains, and its ability to fight free radicals may all change (Aprodu., 2020). Anthocyanins, when subjected to heat processing, are more likely to undergo a variety of processes, such as glycosylation, water nucleophilic attack, cleavage, and polymerization. These reactions all result in the loss of this color and the destruction of the anthocyanin (Rodriguez-Amaya, D. B. 2019). As a result of this, the temperature is another factor that governs the stability of anthocyanin molecular structures; an increase in temperature causes a breakdown of these compounds and influences the degree to which products brown when oxygen is present (Laleh, G. H., et al 2006). (Riaz, M., et al 2016). As a direct result of this, as both time and temperature pass, the color intensity and quantity of monomeric anthocyanins decrease, whilst the number of brown pigments and the polymer part increases. It has been shown that an increase in temperature results in a decrease in the stability of all dietary pigments, including anthocyanins. Concurrently, findings from more recent studies reveal that the chemical structure of these molecules has a direct bearing on their stability, with the sugar fraction having a significant influence in this regard (Hocine et al., 2018). As was discussed before, preventing heat degradation in the anthocyanin solution requires first removing oxygen from the mixture. In point of fact, the combination of a high temperature and the presence of oxygen has been determined to be the most detrimental of all the conditions that may impair the stability of these molecules. (Cavalcanti. et., al 2011). It has been known for a long time that filling the bottles up to the capacity with warm grape juice would slow down the color shift from dark purple to dull brown. Other juices that included anthocyanins produced results that were comparable (Sikorski., 2018). In addition, anthocyanins have the property of being antioxidants because of the reaction that they have with oxygen radicals such as peroxy radicals. This makes them beneficial for the prevention of cardiovascular diseases (Rein., 2005 & Van Hung., 2016). On the other hand, a number of studies have come to the conclusion that the concentration of phenols and anthocyanins will increase during the first few days of cold storage (ranging from zero to seven days) if the food is kept in an environment that contains between sixty and one hundred percent oxygen and a low temperature. However, as more time goes by, this influence will become less significant (Cavalcanti., et al 2011).

3.1.2.2 Oxygen

Due to the fact that their chemical structure is unsaturated, anthocyanins are susceptible to reactions when they come into contact with molecular oxygen (Sikorski, Z. E. 2018). Because of this, oxygen is another essential component that controls the stability of anthocyanins and plays a part in their decomposition. Oxygen can hasten the breakdown of anthocyanins in one of two ways: either through a direct oxidative mechanism or through the action of enzymes that oxidize other molecules (Patras, A., et al 2010). This has a detrimental effect on anthocyanins, and several studies have shown that the stability of these substances may be improved by preserving them not in an atmosphere predominated by oxygen but rather under vacuum, nitrogen, or argon rather than in an environment predominated by oxygen. As a direct result of this, anthocyanin concentrations decreased in each and every environment that was examined, with high oxygen levels causing the greatest decrease (Hocine et al. 2018). As was discussed before, preventing heat degradation in the anthocyanin solution requires first removing oxygen from the mixture. In point of fact, of all the factors that might affect the stability of these compounds, it has been discovered that the combination of a high temperature and the presence of oxygen is the most harmful (Cavalcanti et al. 2011). It has been known for a long time that filling the bottles up to the capacity with warm grape juice would slow down the color shift from dark purple to dull brown. Other juices that included anthocyanins produced results that were comparable (Sikorski,2018). In addition, anthocyanins have the property of being antioxidants because of the reaction that they have with oxygen radicals such as peroxy radicals. This makes them beneficial for the prevention of cardiovascular diseases (Rein 2005 &Van Hung 2016). On the other hand, a number of studies have come to the conclusion that the concentration of phenols and anthocyanins will increase during the first few days of cold storage (ranging from zero to seven days) if the food is kept in an environment that contains between sixty and one hundred percent oxygen and a low temperature. However, as more time goes by, this influence will become less significant (Cavalcanti et al, 2011).

3.1.2.3 Light

Because Light causes an increase in the synthesis and concentration of anthocyanins in plants, Light is an essential component in the anthocyanins' capacity to retain their color (Sikorski, 2018). However, Light has a dual impact on the anthocyanins in the plant. Light is necessary for the production of anthocyanins, but it also speeds up their decomposition (Cavalcanti et al. 2011). The amount of molecular oxygen that is present is another factor that may affect the pace at which light-induced breakdown occurs. It should be underlined that the most severe loss of anthocyanin takes place when pigments are illuminated by fluorescent Light (Hocine et al. 2018). Because anthocyanins are such powerful light absorbers, the orange, red, and blue pigments that are found in a wide variety of plants, including grapes and berries, are due to these pigments. Because of this, the B-ring substitution pattern of the aglycon has a more significant impact on the generation of color than the glycosylation pattern of the flavan structure, which has a less significant influence (Hocine et al. 2018). The effect that Light has on the stability of anthocyanin extracts derived from a broad variety of biological sources has been the subject of a great deal of study throughout the course of history. As a result, it was discovered that around 30 percent of the anthocyanin content in grape juice that had been stored at 20 degrees Celsius in the dark was lost. Despite this, when the same samples were subjected to Light for the same amount of time and at the same temperature, almost half of the total pigments were lost (Amogne et al. 2020). In addition to the research already mentioned, Light may influence the antioxidant activity of anthocyanins. Anthocyanin concentration and antioxidant activity decreased significantly when mulberry fruit extracts were kept at ambient temperature for 10 hours and exposed to fluorescent Light. Longer exposure to Light reduces anthocyanin concentrations and, as a consequence, the extract's antioxidant efficacy (Aramwit et al. 2010). Anthocyanins may be protected by packaging that filters light from the visible spectrum, especially the UV spectrum, and therefore reduces the potentially harmful influence of light on anthocyanins. Additionally, anthocyanins are more resistant to the effects of light because of their glycosylation, acylation, and co-pigmentation (Hocine et al. 2018).

4. Aim

The aim of the study was to see how expedited storage of 40°C for 1-6 months affected polyphenol and flavonol composition and concentrations, as well as overall antioxidants activity in various freeze-dried berries. The muesli specimens were taken out of freeze-dried strawberries, black currant, or blueberry made under subsection in Estonia.

5. Materials and Methods

5.1 Materials

5.1.1 Chemicals: Cyanidin 3- glucoside was purchased from. Methanol and Acetonitrile were of HPLC grade. Formic acid was of analytical grade. Methanol and water of gradient grade for liquid chromatography were purchased from Merck KGaA (Darmstadt, Germany). Ethyl acetate of analytical grade was purchased from.

5.1.2 Standards: The 3-O-â-glucoside standards of pelargonidin, cyanidin, peonidin, petunidine, delphinidin, and malvidin (six mixed anthocyanin standards, HPLC grade) and cyanidin 3-glucoside (HPLC grade) were obtained from Polyphenols Laboratories (Sandnes, Norway).

5.1.3 Berry Samples: Berries samples were collected Musli packages including freeze dried strawberry, blackcurrant and bluberry material prepared in in Estonia. Mainly berries were isolated from the Musli. Inside the musli there were pinuts and berries which fully covered by full cream powder milk. As shown below in the image unopen at left side and open packet at right.

5.2 Methodologies

Different freeze-dried berries were used in this study and maintained at accelerated storage of 40 °C for a period of one to six months. HPLC-DAD method for simultaneous determination of natural polyphenols and HPLC-MS instruments were used to identify and quantify the phenolic compounds in the berries.

5.2.1 Extraction process for anthocyanins samples

The extraction were performed in 4 replicate. Isolated freeze dried berries were ground into a powder and weighed (1.00 g). Then 3 ml of extraction solution was added. The extraction solution consisted of 1% solvent A and 99 percent solvent B (Meoh/Hcl 99:1 v/v). The samples were vortex for 1 min and sonicated for 10 min. Centrifuged 3000 rpm for 10 mins. Supernatants were collected to 50 ml volumetric flask. This process were repeated three times and then supernantents were combined and dilute with extraction solvents where the total volume was adjusted to 50 ml. All extracts were filtered through 0.22mm PTFE filter (vwr international, France) prior to HPLC analysis.

5.2.2 Extraction process for flavonols and other phenolic samples

For extraction, 1.00 g of freeze dried berris were ground into powder. 10ml of extraction solvents was added which contain ethyl acetate. Vortex for 1 min and the extracts were sonicated for 15 min in ultrasonic bath (Origin). Centrifuged for 15 min at 3000 rpm. After that supernatants were collected in 250 ml flat bottom flask. This process was repeated four times and supernatants were combined. All supernatants were evaporated at 37-degree C to completely dry and residue were dissolved in 3ml Methanol (MeoH). Extracts were filtered through 0, 22 mm PTFE filter (vwr International, France) prior to HPLC Analysis.

5.2.3 HPLC-DAD analysis of anthocyanins

The high-performance liquid chromatography-diode-array (HPLC-DAD) detection instrument consisted of a Shimadzu (Shimadzu Corporation, Kyoto, Japan) SIL-20AC auto sampler, a sample cooler, two LC-20AD pumps, a CTO-20AC column oven, an SPD-M20A diode array detector and a CBM-20A central unit. It's from the Phenomenex Company). A 150 x 4.6 mm and 3.6 micrometer peptide XB-c18 column used for separation. Elution were performed using mobile phase A (5% formic acid in Milli-Q water) and mobile phase B (Acetonitrile).

The system was operated using Lab Solutions Workstation software. Flow rate of the mobile phase was 1 ml/min, and the injection volume was 10 µl and peaks were monitored at 520 nm with a DAD.

For quantification and identification purposes, a mixture of six anthocyanins standards were used to create a calibration curve of the individual anthocyanins. The result were expressed as anthocyanins glucodise equivalents.

For the analysis of falovonols and other phenolics, elution were performed using mobile phase A (0.1% formic acid in Milli-Q water) and mobile phase B (0.1% formic acid in Acetonitrile).

5.2.4 Statistical Analysis

The quantitative analysis of chemical compounds was carried out in triplicate. The results were calculated using dry weight (mg/100g of berries) and expressed as mean ± standard deviation (SD).

6. Result and Discussion

6.1 Storage stability of Freeze-dried items

Technology has evolved, and the ways of consumption and storage of food have changed as well. There are various methods to preserve food and keep it fresh for the longest time. The process of preserving food is so diverse and modified to preserve food items in their natural color. Food is naturally in different colors. Anthocyanin is the pigment that normally gives food red, blue, purple or any rich color to plants, and they are strong antioxidants; they also act as anti-inflammatory, anti-viral, and anti-cancer as well are helpful in prevention from these diseases, and these have high nutrition value as well. The storage stability of these materials is not very complex as these foods have phenolic compounds. Phenolic compounds are present in various food items such as coffee, tea, fruits, vegetables, chocolates, wine, honey, and it is present in oil as well; and these compounds have chemical structure compromising aromatic rings that has antioxidant properties. Berries contain a huge amount of Anthocyanin that is supremely beneficial in pertaining to chronic disease, and it also has the power to boost immunity, so berries are more likely to be used and consumed on a larger scale. The berries are frozen or dried up to be used throughout the year. The process of using preserving these berries can be risky, and it can degrade the nutritional value of the food. Therefore, it is important to get away with the methods that can retain its color and also maintain the nutrition inside the fruit so it can boost the immune system and help the person to have a better immune system so that they can prevent any chronic disease and remain healthy for the longest time.

6.2 Preservation of fruits

Phenolic compounds and rich in boosting the immune system are most likely to be found in every plant, and these are supremely helpful in boosting the immune system and saving people from various diseases. As people have got to know about the benefits of consuming berries, different companies introduced different methods to prevent these fruit that is so rich in nutrition. The process of preservation is very diverse, but the most common method that is being used to preserve the food is the freeze-drying method. The particular method that is used to freeze berries starts by freezing the fruits initially. When the fruits are completely frozen, then these frozen items are kept under vacuum, and when the frozen items are under low pressure, the ice crystal sublime and remove water very efficiently. The process is very productive, and it increases the life of these fruit items. The most important question that arises is that the most important antioxidant is still there in the food with full power, and it will be as useful as we attain it from fresh fruits. The method is so profound and very useful to be used to retain the nutrition of the fruits. Vegetables and fruits are supremely useful in making our body healthy and boosting our metabolism, so it is mandatory to lock the nutrition for the longest time. The freeze-dried method has been used in preserving food items for the longest period of time. Anthocyanin is the most precious element that has to stay inside the berries, and people can consume these fruits and vegetables for a good time span.

6.3 Storage effects on Anthocyanin

Consumers are very well aware of the nutritional value of these berries, and most of the people are into workouts and exercise at the gyms, so in their tough routines, they get intricate diet plans, and people use mueslis and granolas, so the nuts and these dried berries are used in these food items. We have conducted some tests for 120 days. We used three different berries to conduct the test of whether or not these food items will lose an element like Anthocyanin. The test has been conducted in a laboratory, and the food items were observed keenly as these food items have lost a particular percentage of nutrition. The food items that are being used to conduct the test are Strawberries, Blueberries, and Blackcurrant. These berries were taken from the packets of mueslis and kept under consideration for 120 days. Food is the fuel of the human body, and we must eat food items that are high on nutrition. The food items are supposed to be validated and determined under the HPLC-DAD methods that are specifically used to evaluate whether the food items are preserved properly, and these instruments are used to evaluate that the six phenolic acids in the aqueous extract and it is used to evaluate the concentration range in the food items as well. The HPLC-MS is also a system that is intricately designed to examine the particular food item that is present in these frozen-dried food items with the appropriate nutrition, and the food items are still very useful for the person who is going to buy these preserved food items and use them for attaining appropriate benefit.

6.4 Result and evaluations of the test conducted on berries for 120 days

The experiment has been conducted for 120 days, and the results are appropriate and authentic that is helpful to evaluate the nutrition in the food items. These food items are also evaluated on the perimeters set by the sensitive, high-performance liquid chromatography methods that are used to evaluate that these food items might not conduct any item that can cause any disease in the human body, and they have not changed the phenolic compound into something hazardous. The authentic and appropriate methods are very modern and accurate to evaluate the constraints that are supremely beneficial for the human body. The three berries kept under observation for 120 days, and we evaluated that how much Anthocyanin has been lost from the particular food items. The results are described in the different pictures and graphs as well, so will describe all the details that are conducted during the experiment. First evaluate the tables, graphs, and pictures about one fruit and will analyze the ratio of the oxidants that are still present in the food and how much we have lost. The analysis was from day 0, then the analysis on day 28, 56, 89 and the last analysis is conducted on day 120, and evaluate the result based on the particular result.

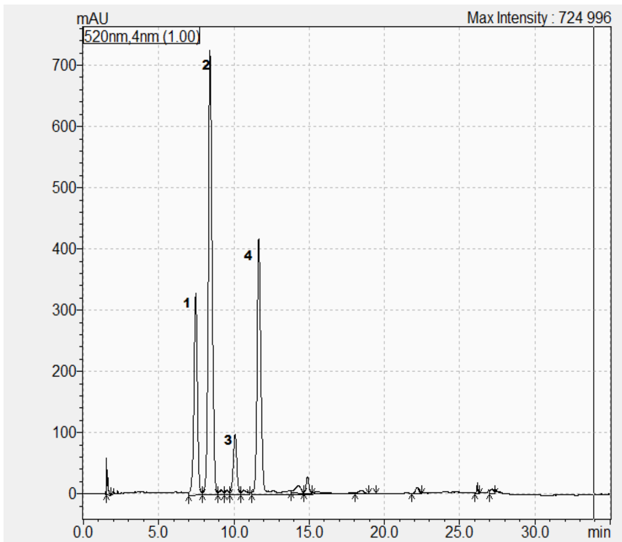
6.4.1 Identification and quantification of anthocyanins in freeze dried Blackcurrant, Blueberry and strawberry

The aim of the HPLC method optimization was to find the most suitable condition for the determination of major anthocyanin occurring in blackcurrant, blueberries and strawberries. The main requirements for the HPLC analysis was the efficient separation of major anthocyanins and further minor anthocyanins with sufficient resolution and narrow peaks in a short time. The wavelength chosen for anthocyanin detection was 520 nm. Other colored compound visible at 520 nm were assumed to be minor undefined anthocyanins.

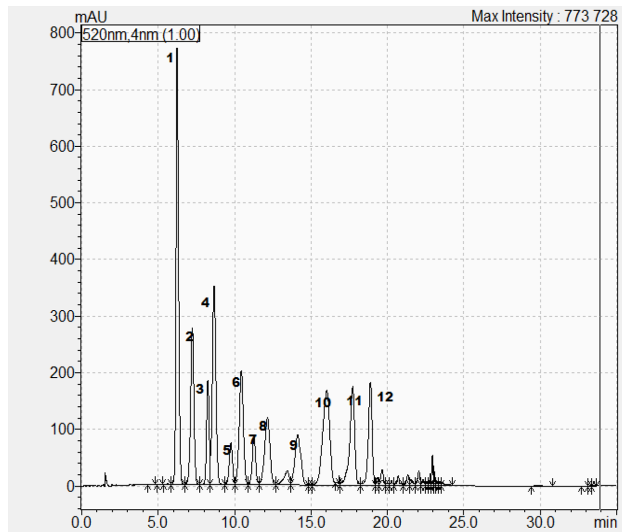
Identification and peak assessment of anthocyanins were based on the comparison of their retention time and with standard and published data. Under current separation condition, the elution order of six mixed anthocyanins delphinidin3-glucoside, cyaniding 3-glucoside, petunidin 3-glucoside, pelargronidin 3- glucoside, peonidin 3- glucoside and malvidin 3- glucoside. Quantification of anthocyanins was performed by utilizing the corresponding anthocyanins 3-glucoside as a standard (Table 1). Peaks **(Figure 6**) were identified with literature (Zheng & Wang, 2003): cyanidin 3-galactoside, cyanidin 3-arabinoside, peonidin 3-galactoside and peonidin 3-arabinoside.

Black Currant has anthocyanin profile. A representative chromatogram of anthocyanins from black currant has shown in **Figure 6 (A).** Anthocyanins were detected by comprising their MS data. Under current separation condition, the retention time of delphinidin 3-arobinoside should be shorter than that of cyaniding 3- glucoside. The sequence of elution of anthocyanins glycosides under our condition is galactose, glucose, arabinose and xylose. Data on the concentration of anthocyanins in blackcurrant id much higher. Its might be for different pH method.

(A)



(B)



(C)

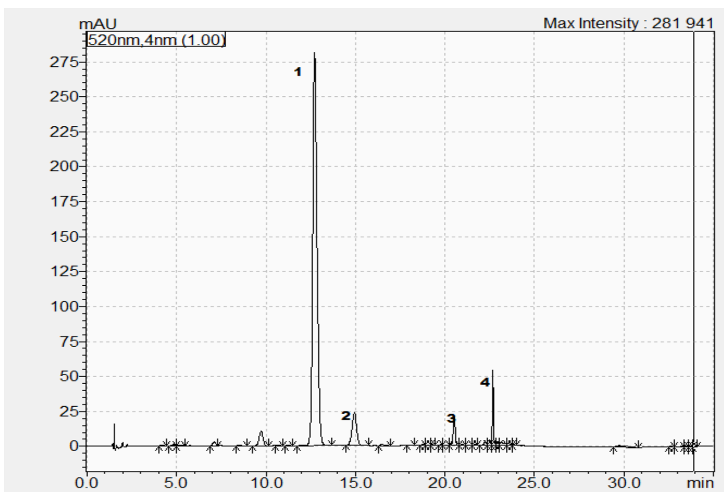


Figure 6 (A), (B), (C): HPLC-DAD chromatogram at 520 nm of (A) Blackcurrant, (B) Blubbery and (C) Strawberry.

The experiment has explained the ratio abruptly in the graph is chromatogram at 520 nm of Blackcurrant **Figure 6 (A).** Delphinidin galactoside reached 300. Delphinidin rutinoside reached 700; delphinidin glucoside reached 100; Cyanidin Rutinoside reached 400. These show that how important elements are reached till particular ratio that affects the nutrition value of the fruit. In the same way Figure 6 (B) and (C) showed the particular ratio.

Blueberry contain a lot of important compounds and nutrients that are lost after a particular time span. The validation from HPLC-DAD **Figure 6 (B)** displays result that shows that Delphinidin galactoside has reached 800, delphinidin glucoside reached above 250, Cyanidin galactose has reached 190. Delphinidin arabinoside has reached above 350; Cyanidin glucoside reached 190, Petunidin galactoside 80. Cyanidin arabinoside is around 100.

As we have attained the validation from the HPLC-DAD chromatogram at 520 nm of strawberry **Figure 6 (C)** Peak identification: 1. Pelargronidin rutinoside 2. Pelargronidin glucoside 3. Cyanidin glucoside shows that Pelargronidin rutinoside is above 275, and the element like Pelargronidin glucoside is at 15, and the last one is Cynaidin Glucoside is at 50.

Table 3: Identification of anthocyanins from freeze dried Blackcurrant, Blueberry and Strawberry sample

Sample		Retention time	Compounds
Blackcurrant	1	7.451	Delphinidin 3-O-glucoside
	2	8.399	Delphinidin 3-O-rutinoside
	3	10.074	Cyanidin 3-O-glucoside
	4	11.642	Cyanidin 3-O-rutinoside
	5	14.882	Delphinidin
	6	22.173	Cyanidin
Blueberry	1	6.659	Delphinidin 3-O-galactoside
	2	7.744	Delphinidin 3-O-glucoside
	3	8.797	Cyanidin 3-O-galactoside
	4	9.259	Delphinidin 3-O-arabinoside
	5	10.426	Cyanidin 3-O-glucoside
	6	11.172	Petunidin 3-O-galactoside
	7	12.01	Cyanidin 3-O-arabinoside
	8	12.994	Petunidin 3-O-glucoside
	9	14.297	Peonidin 3-O-galactoside
	10	15.039	Petunidin 3-O-arabinoside
			Delphinidin
	11	17.051	Malvidin 3-O-galactoside
	12	19.594	Malvidin 3-O-glucoside
	13	22.211	Malvidin 3-O-arabinoside
	14	22.528	Cyanidin
	15	23.544	Delphinidin 3-O-(6''-acetyl)-glucoside
	16	24.313	Petunidin
	17	25.321	Petunidin 3-O-(6''-acetyl)-glucoside
	18	25.574	Malvidin 3-O-(6''-acetyl)-galactoside
	19	26.427	Malvidin 3-O-(6''-acetyl)-glucoside

	20	26.546	Malvidin
Strawberry	1	9.72	Cyanidin 3-O-glucoside
	2	12.71	Pelargonidin 3-O-glucoside
	3	14.94	Pelargonidin 3-O-rutinoside
	4	20.52	Pelargonidin 3-O-(6"-malonyl)-glucoside
	5	22.238	Pelargonidin
	6	22.68	Pelargonidin 3-O-(6"-succinyl)-glucoside

6.4.2 Storage stability of black currant in 120 days

The reduction in the particular berries is explained in the tables and graphs very particularly after experimenting for 120 days.

Table 4: Anthocyanin contents in Blackcurrant in different days (Mg100/g, Dry weight, ± Standard deviation, SD):

Compounds	0 Days	28days	56 Days	89 Days	120 Days
Delphinidin 3-O-glucoside	48.3±61.2	31.3 ± 3.0	27.8 ±2.8	11.8 ± 1.2	12.4 ± 9.9
Delphinidin 3-O-rutinoside	132.6±17	82.5 ±7.9	73.0 ±9.5	34.1 ±5.7	32.7± 22.9
Cyanidin 3-O-glucoside	17.8 ±1.9	16.2 ±2.3	12.3± 12.2	10.1± 3.2	6.8 ± 5.9
Cyanidin 3-O-rutinoside	87.0 ±8.6	47.6 ±15.6	39.2± 9.0	38.6± 7.0	33.1 ± 26.9
Delphinidin	2.2± 0.4	3.5 ± 0.5	1.8 ± 0.3	2.8 ± 1.0	2.3 ± 2.0
Cyanidin	1.0 ± 0.2	3.1 ± 0.5	1.1 ± 1.0	2.4 ± 0.7	7.2 ± 11.7

The **table 4** detailed how the reduction occurred in specific components. The deviation displays ratios. Delphinidin 3-0-glucoside attained 12.40±9.9, Delphinidin 3-0-rutinoside attained 32.7± 22.9, Cyanidin 3-0-glucoside attained 6.8, and Cyanidin 3-0-rutinoside attained 33.1. The deviation has properly clarified the elements. The table shows that different anthocyanin components deteriorate in varying amounts over time. Some of them shrank less over time, while others shrank more.

6.4.3 Storage stability of blueberry in 120 days

The sample that has been kept under consideration is blueberries. The reduction in the particular berries is explained in the tables and graphs very particularly after experimenting for 120 days

Table 5: Anthocyanin contents in blueberries in different days (Mg/100g, Dry weight, ± Standard deviation, SD)

Compounds	0 Days	28days	56 Days	89 Days	120 Days
Delphinidin 3-O-galactoside	63.8± 9.2	40.2 ±8.4	31.2 ± 3.3	20.1± 3.8	13.7 ± 2.3
Delphinidin 3-O-glucoside	31.6±3.1	16.4 ± 2.3	14.2 ± 1.7	8.6± 1.1	6.6 ± 0.8
Cyanidin 3-O-galactoside	33.6±2.1	16.2 ± 10.5	13.5 ± 1.0	9.0 ± 1.4	9.1 ± 1.5
Delphinidin 3-O arabinoside	43.2 ±2.1	30.0 ± 5.4	21.2 ± 1.9	9.2 ± 5.4	13.7 ± 21.5
Cyanidin 3-O-glucoside	17.8±3.7	9.1 ± 1.7	6.5 ± 0.9	4.5 ± 0.8	13.1± 2.2
Petunidin 3-O-galactoside	49±19.4	37.8± 8.0	27.3± 3.9	17.5 ± 3.8	3.7 ± 0.4
Cyanidin 3-O-arabinoside	21.0±0.7	14.3 ± 3.2	8.5± 0.4	5.1 ± 0.7	9.7 ± 0.9
Petunidin 3-O-glucoside	36.9±8.9	24.5± 3.0	19.9 ± 1.5	12.0 ± 1.5	4.3 ± 3.9
Peonidin 3-O-galactoside	4.1±0.7	2.8 ± 0.8	1.6 ± 0.2	1.0 ± 0.1	7.2 ± 1.5
Petunidin 3-O-arabinoside	16.1±5.5	17.1± 4.9	13.9 ± 2.0	7.5 ± 2.7	1.4 ± 0.2
Delphinidin	9.2±1.0	6.3 ± 0.7	3.8 ± 0.5	2.7 ± 0.7	35.1± 4.9
Malvidin 3-O-galactoside	308.6 ± 414	153.2 ± 27.5	61.7 ± 9.4	39.0 ± 2.9	20.9 ± 2.5
Malvidin 3-O-arabinoside	191.8 ± 252	90.4 ± 15.3	39.8± 3.2	19.6 ± 2.5	1.0 ± 0.3
Cyanidin	9.7 ± 2.9	4.2 ± 0.8	33.9 ± 4.3	1.2 ± 0.3	1.0 ± 0.3

Delphinidin galactoside and glucoside have been lowered to 13.7 and 6.6, respectively. Cyanidin 3-0-galactoside is at 9.1, Delphinidin 3-0- arabinoside is at 14.83, Cyanidin 3-0-glucoside is at 21.6, Petunidin 3-0-galactoside is at 13.7, and Cyanidin 3-0- arabinoside is at 9.7. These nutrients are reduced to a specific ratio, and their value declines until the ratio depletes the fruit's natural power. Malvidin arabinoside and glucoside levels were particularly low at the end of 120 days, at 20.9 and 1.0, respectively. According to the table, all anthocyanin components diminish day by day during storage.

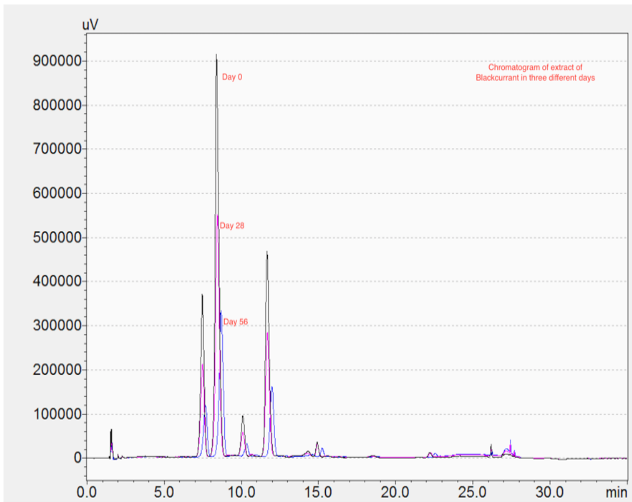
6.4.4 Storage stability of strawberry in 120 days

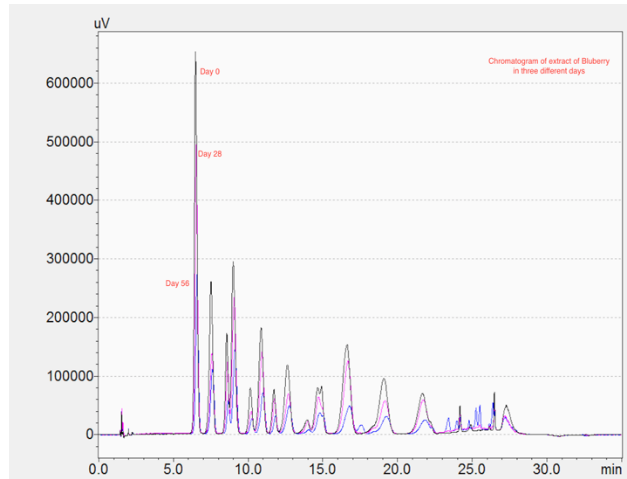
Table 6: Anthocyanin contents in strawberries in different days (Mg/100g, Dry weight, ± Standard deviation, SD):

Compounds	**0 Days**	**28days**	**56 Days**	**89 Days**	**120 Days**
Cyanidin 3-O-glucoside	5.2±5.4	1.5 ±0.8	1.1 ±0.4	2.2± 3.8	0.8 ±0.5
Pelargonidin 3-O-glucoside	100±54	38.6 ±4.8	34.8 ±1.2	18.3± 1.8	17.3 ±3.6
Pelargonidin 3-O-rutinoside	7.3±1.9	2.5 ±0.5	2.5 ±1.2	1.3 ±0.1	1.1 ±0.1
Pelargonidin 3-O-(6"-malonyl)-glucoside	5.5±2.5	2.7 ±0.7	3.5 ±0.9	2.1 ±0.3	1.6± 0.3
Pelargonidin	1.2±0.0	0.9 ±0.0	0.9 ±0.0	0.8 ±0.0	0.8 ± 0.0
Pelargonidin 3-O-(6"-succinyl)-glucoside	7.3±3.0	2.8 ± 0.5	1.8 ±0.3	1.8 ±0.2	1.8 ±0.3

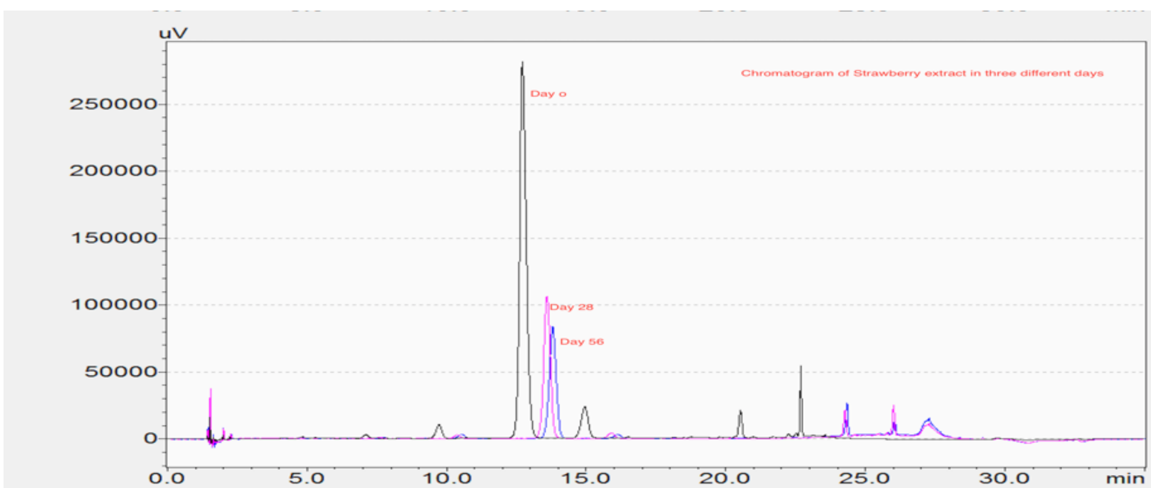
Like the other fruits in the previous table, Strawberry loses its anthocyanin concentration over time. From day 0 to day 120, the ratio shows how pelargonidin glucoside and rutinoside decreased. The element was 100 and 7.3 mg/100gm on day 0, but by day 120, it had dropped to 1.1 and 1.6 mg/100gm, indicating that the Anthocyanin element is still present in the food item as it approaches day 56 of prevention, which is quite low.

6.4.5 Comparison of peak height in different days from the validation of HPLC-DAD:





(A) **(B)**

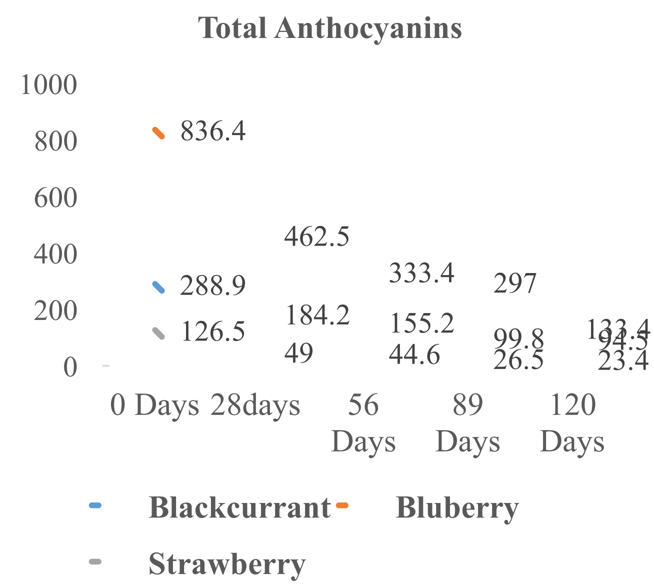


(C)

Figure 7 (A) (B) (C): Chromatogram of Blackcurrant (A), Blueberry (B) and Strawberry (C) extract in different days.

Figure 7 (A), (B), and (C) show how the reduction was initially slow, but when the food items remained for more than 40 days, the reduction of Anthocyanin was very rapid. The average reduction on the 28th day was 45 percent, and by day 56, it had dropped to 56 percent. The study demonstrates how food items lose nutrition, even when we use the most diverse and advanced methods to preserve them.

6.4.6 Degradation of Total anthocyanin



Sample	0 Days	28 Days	56 Days	89 Days	120 Days
Blackcurrant	288.9	184.2	155.2	99.8	94.5
Blueberry	836.4	462.5	333.4	297	133.4
Strawberry	126.5	49.0	44.6	26.5	23.4

Figure 8: The line graph shows the total anthocyanin losses after 120 days. Anthocyanins in blueberries decreased by half between 0 and 56 days, and by 120 days, they were nearly six times lower than at the start. Similarly, anthocyanins in blackcurrant were reduced by 46.27 percent after 56 days and 39.11 percent after 120 days. After 120 days, the degradation trend in strawberries is nearly identical.

6.4.7 Comparison with other related study

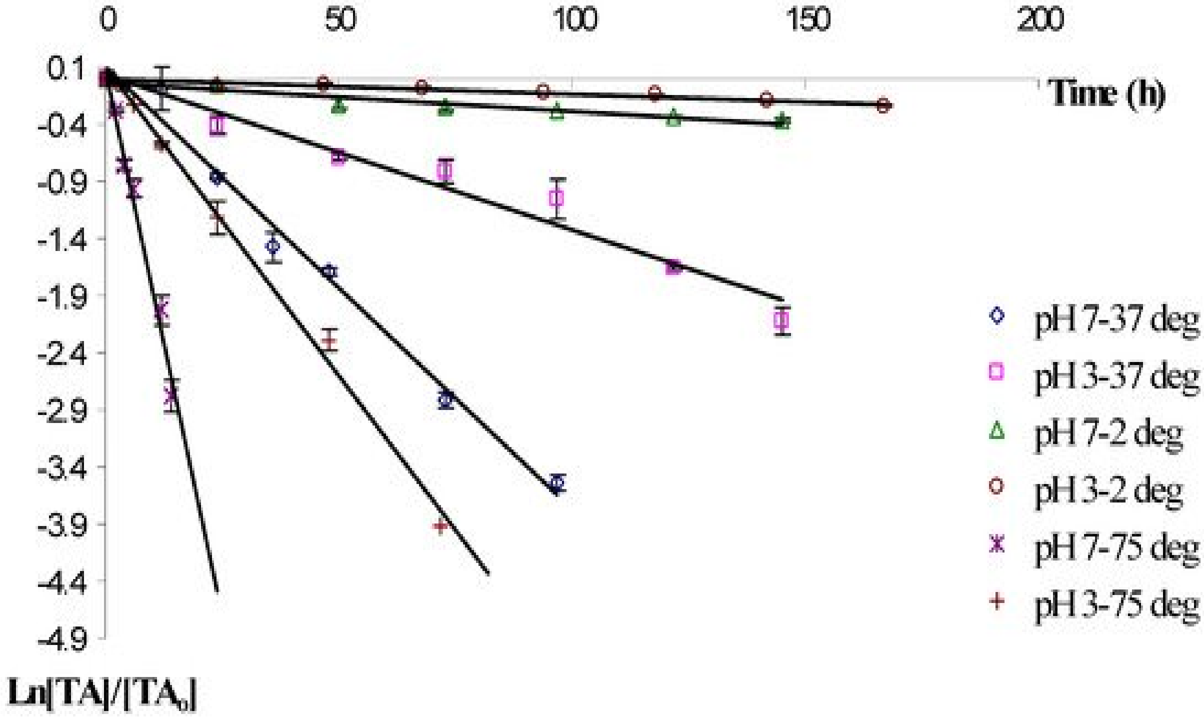


Figure 9: Anthocyanin degradation in cranberry bush fruits aqueous extract during storage at various temperatures and pH levels (vertical lines represent SD, n = 3).

Several studies (**figure 9)** have revealed that the storage temperature has a significant impact on the degradation rate of anthocyanins. When compared to refrigerated storage at 2 °C, storage at 37 °C resulted in faster degradation (Moldovan 2012). In comparison to other studies, the decreasing trend of anthocyanin in this study is quite similar.

7. Conclusion

Some of the richest sources of polyphenols are berries. These micronutrients that naturally occur in plants are seen to help with the reduction of the potential risk of chronic diseases. However, polyphenols such as Anthocyanin are very susceptible to degradation during processing and storage. Anthocyanin is present in fruit and vegetables and has been receiving increased attention because of its potential antioxidant activity. While the level of Anthocyanin can be significantly high in fresh berries, it degenerates remarkably when the berries are processed or stored.

The experiment conducted on the food items explained that how the food losses nutrition after 60 or more days. We are unable to attain the nutrition that is attained by conducting fresh food. These experiments have explained the procedures profoundly. The storage stability under the most efficient efforts only leaves 50 percent nutrition and effective compounds in food items. After two months of storage (56 Days), there were significant losses in anthocyanins in all berries. Strawberries recorded as much as 60%, 60% in blueberries, and 46% in blackcurrant. These losses were in comparison to the amount found in the berries on day zero. Moreover, after 120 days, anthocyanin losses were quite low from 56 days e.g. 21% in strawberry, 59% in blueberry, and 29% in blackcurrant. Contrary enough, the variety of flavonols detected and quantified with HPLC from the samples remained unchanged during the first months of storage. The result if this research still provided evidence on the degradation kinetics of individual as well as total anthocyanin in different berries when stored at 40°C temperature for long period. The individual anthocyanin exhibited different degradation rates, which might be related to their chemical composition. Higher temperature and long storage periods not only reduced anthocyanin concentration but also change the color as well.

Therefore, the findings could be adapted to industrial application for preserving the biologically active anthocyanin by storing them at low temperature for limited storage periods. So, time is the additional challenge in this case to develop product.

More research is needed in the future to determine how long anthocyanins present in fruits. If they are stored for more than 6 months and to identify the specific factor responsible for anthocyanin degradation during storage time.

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