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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

Dissertação

SEPARAÇÃO E PURIFICAÇÃO DE COMPOSTOS DE ANTOCIANINA A PARTIR DO RESÍDUO DA FERMENTAÇÃO DE CASCA DA UVA *ALICANTE BOUSCHET* UTILIZANDO RESINAS MACROPOROSAS

SIRAJ SALMAN MOHAMMAD

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Sob a orientação do professor
José Lucena Barbosa Junior

Dissertação submetida como requisito parcial para obtenção do grau de **Mestre em Ciência e Tecnologia de Alimentos**, do programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, área de concentração em Ciência de Alimentos.

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RESUMO

MOHAMMAD, Siraj Salman. **Separação e purificação de compostos de antocianina a partir do resíduo da fermentação de casca da uva variedade *Alicante Bouschet* utilizando resinas macroporosas**. 2019. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2019.

As uvas são uma das culturas frutíferas mais comumente produzidas no mundo. Consequentemente, a indústria da uva produz grandes quantidades de bagaço, que é uma fonte rica em polifenóis que promovem a saúde, como proantocianidinas, flavonas e flavan-3-ols. Neste contexto, existem muitos tipos de adsorventes disponíveis, como as resinas macroporosas (MARs), as quais são relatadas como sendo mais eficiente para a recuperação de polifenóis devido às suas características e por apresentarem baixo custo, alta capacidade de recuperação e longo prazo de validade. Portanto, neste estudo, a matéria prima utilizada foi o resíduo da fermentação de casca de uva *Alicante Bouschet* da produção de vinho tinto, (1 g) de bagaço de uva seco foi pesado e extraído com 12,5 mL de 50% de água e 50% de etanol, o resíduo foi extraído novamente pelo mesmo volume de solução, as características de adsorção desorção de antocianinas de resíduo da fermentação de casca da uva *Alicante Bouschet* (RFCU) em seis tipos de resinas macroporosas (XAD 2, XAD 4, XAD 7HP, XAD 8, XAD 11 e XAD 16) foram investigadas. Nos testes de adsorção estáticos, XAD 7HP e DAX 8 apresentaram os melhores desempenhos para a antocianina total (AT). Os estudos das isotermas indicaram que o mecanismo de adsorção foi melhor explicado pelo modelo de isoterma de Langmuir. Por sua vez, as cinéticas de adsorção foram melhor explicados pelo modelo de pseudo-segunda ordem. O processo de adsorção utilizando as resinas XAD 7HP e DAX 8 foram isotérmicos nas temperaturas avaliadas (30 °C, 40 °C e 50 °C). Os extratos purificados apresentaram alta capacidade antioxidante acessadas pelos métodos FRAP e DPPH. Em relação à análise por cromatografia líquida de alta eficiência (CLAE), Peonidina, Peonidina-3-glicosídeo acilada, malvidina acilada e malvidina-3-glicosídeo foram os quatro principais compostos antociânicos tanto no extrato inicial quanto no purificado. No geral, as resinas macroporosas demonstraram potencial como alternativa na purificação e separação de antocianinas de resíduo de vinícolas, ao uso solventes orgânicos.

Palavras-chave: purificação, polifenóis, extração, antioxidante.

ABSTRACT

MOHAMMAD, Siraj Salman. **Separation and purification of anthocyanin compounds from grape wine pele pomace (*Alicante Bouschet*) using macroporous resin**. 2019. Dissertation (Msc in Food Science and Technology). Instituto de Tecnologia, Departamento de Tecnologia de Alimentos, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2019.

Grapes are one of the most commonly produced fruit crops in the world. Consequently, the grape industry produces large quantities of grape pomace which is a rich source of health promoting polyphenols such as proanthocyanidins, flavones and flavan-3-ols, in this context, there are many types of adsorbents available, but macroporous resins are reported to be the most efficient for polyphenol recovery because of its characteristics like low cost, high recuperative capacity and long term-validity. Therefore; in this study, the raw material used was the residue from grape fermentation of *Alicante Bouschet* from the production of red wine, (1 g) of dry GP was weighed and extracted with 12.5 mL of 50% water and 50% ethanol, the residue was extracted again by the same volume of solution, the adsorption/desorption characteristics of grape wine peel pomace (GWPP) anthocyanins on six types of macroporous resins (XAD 2, XAD 4, XAD 7HP, XAD 8, XAD 11 and XAD 16 were investigated. On basis of static adsorption test, XAD-7HP and DAX 8 showed higher adsorption/desorption capacities. The adsorption mechanism indicated that the process was better explained by pseudo-first-order kinetics and the Langmuir isotherm model. Adsorption isotherm tests showed that the adsorption process was exothermic on XAD 7HP and DAX 8 when performed at 30° C, 40 °C and 50 °C. The results of antioxidant tests indicate that purified anthocyanin extract showed high antioxidant capacity in the both methods of FRAP and DPPH Regarding to high-performance liquid chromatography analysis, acylated Peonidin, Peonidin-3-glucoside, acylated malvidin and malvidine-3-glucoside are the four main anthocyanin compounds in the initial and purified extract of GWPP. Overall, macroporous resins can be an effective tool to purificate anthocyanin instead of using organic solvents and supply pharmaceutical and food industry with safe and cheap process.

Keywords: purification, polyphenol, extraction, antioxidant

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LISTA DE ABREVIATURAS

1/n	Constante empírica do modelo / empirical constant
3-MA	3-methyladenine / 3-metiladenina
A	the adsorption ratio / taxa de adsorção
AAT	atividade antioxidante total
AG	ácido gálico
AGE	ácidos graxos essenciais
AGPIS	ácidos graxos poli-insaturados
AMA	aornia melanocarpa anthocyanin
BU	bagaço de uva
Cd	the concentration of total anthocyanin in the desorption solution (mg/L)- a concentração de antocianina total na solução de dessorção (mg / L)
Ce	the equilibrium concentration of total anthocyanin in the extracts / a concentração de equilíbrio da antocianina total nos extratos-
CQ	Chloroquine / cloroquina
D	desorption ratio (%)-razão de dessorção (%)
Df	dilution fator / fator de diluição
DO	destilação osmótica-
ECSA	extração com solvente acelerado
EFP	extração com fluido pressurizado
EFS	extração com fluido supercrítico
eNOS	endothelial-type nitric oxide synthase / sintase do óxido nítrico do tipo endotelial
ERC	espécies reativas de cloro-
ERN	espécies reativas de nitrogênio
ERO	espécies reativas de oxigênio
ESA	extração subcrítica de água
EXP	experimental values / valores experimentais
FSU	Fenólicos de semente de uva- Grape seed phenolics
FVs	flavylium salts- sais de flavio
GWPP	grape wine peel pomace / bagaço de casca de vinho de uva
HCA	hydroxycinnamic acid / ácido hidroxicinâmico
HCASMC	human coronary artery smooth muscle cells / células musculares lisas da artéria coronária humana

K	constant of Henry model / constante do modelo de Henry.
KF	the Freundlich constant / a constante de Freundlich
Kl	constant of Langmuir model / constante do modelo de Langmuir
Ks	the rate constant of the pseudo-second-order-model / a constante de velocidade da pseudo-segunda-ordem-
LDL	low-density lipoprotein / lipoproteína de baixa densidade
LPS	Lipopolysaccharide / Lipopolissacarídeo
M	the initial weight of resin / o peso inicial da resina
MA	mulberry anthocyanins
MAE	Microwave assisted extraction- Extração assistida por micro-ondas
MF	Microfiltration / microfiltração
Mod	pseudo-second-order model values / valores do modelo de pseudo-segunda ordem
Mw	molecular weight / peso molecular
NO	nitric oxide / óxido nítrico
PAD	photodiode array detector / detector de arranjo fotodiodo
PI3K	phosphoinositide 3-kinase / fosfoinositido 3-quinase
p-JNK	phospho-c-Jun N-terminal Kinase 1-
PKC	protein kinase C
Q	adsorption capacity / capacidade de adsorção
Qe	adsorption capacity at equilibrium / capacidade de adsorção em equilíbrio
qm	the maximum adsorption capacity / a capacidade máxima de adsorção
R	the recovery of total anthocyanin % / a recuperação de antocianina total% -
RCS	reactive chlorine species / espécies reativas de cloro
RFCU	resíduo da fermentação de casca da uva
RNS	reactive nitrogen species
ROS	reactive oxygen species
SFA	ácido graxo saturado
T	Time / tempo
TA	Total anthocyanin / Antocianina total
TAC	Total anthocyanin contentes / Teor total de antocianinas
Te	Trolox equivalent / Equivalente Trolox
TMA	Total monomeric anthocyanin / Antocianina monomérica total
Tr	Total anthocyanin contentes / Teor total de antocianinas

UF	Ultrafiltrarão
UV	ultra violet
Vd	the volume of the desorption solution (mL) / o volume da solução de dessorção (mL)
WRA	wine residues anthocyanins / resíduos de vinho antocianinas
Co	initial concentration of total anthocyanin in the extracts / concentração inicial de antocianina total nos extratos
Kf	the rate constant of the pseudo-first-order-model / a constante de taxa do modelo de pseudo-primeira ordem

SUMÁRIO

ESTRUTURA DA DISSERTAÇÃO

A dissertação está estruturada conforme descrito a seguir: No Capítulo I, é apresentada uma revisão de literatura acerca dos principais artigos científicos abordando as propriedades mais importantes das antocianinas; efeito antioxidante, saúde e benefícios nutricionais. Além disso, seu potencial de uso terapêutico e profilático na saúde dos consumidores.

No Capítulo II, são apresentados resultados concernentes ao estudo da extração e purificação de compostos antocianinas a partir de resíduo da fermentação de casca da uva (RFCU) utilizando 6 diferentes resinas macroporosas de adsorção, onde foram realizados testes de adsorção e dessorção estáticos, avaliando-se as diferentes resinas e; cinéticas de adsorção; isotermas de adsorção, avaliando-se diferentes temperaturas de adsorção e diferentes concentrações dos extratos; além de avaliação da capacidade antioxidante de extrato inicial e extrato purificado por XAD 7HP; e por fim, comparação do perfil de compostos fenólicos das amostras secas e dos extratos purificados.

Cada capítulo está apresentado na forma de artigo e, portanto, está formatado de acordo com as normas exigidas por cada revista à qual foi submetido.

1. INTRODUÇÃO GERAL

As uvas são uma das culturas frutíferas mais abundantes no mundo, com aproximadamente 75 milhões de toneladas produzidas a cada ano e 80% desta produção usada para fazer vinho, o restante é consumido como fruta (fresca ou desidratada) ou sucos (concentrado ou não) (Fontana, Antoniolli et al. 2013, Moncayo and Aurand 2016). Portanto, a indústria da uva gera grandes quantidades de bagaço que possuem expressivas quantidades de fitoquímicos promotores de saúde como proantocianidinas, flavonas e flavan-3-ols; flavonóides (quercetina, kaemferol, miricetina), catequinas ou fenóis (carnosol, rosmanol, rosamaridifenol) e ácidos fenólicos (ácido carnósico, ácido rosmarínico) (Cheynier and Rigaud 1986).

Os fabricantes de alimentos têm usado antioxidantes relativos aos alimentos, principalmente de natureza fenólica como antocianinas, para evitar a deterioração da qualidade dos produtos e manter seu valor nutricional. Antioxidantes também têm sido de interesse para profissionais de saúde porque ajudam o corpo a se proteger contra os danos causados por espécies reativas de oxigênio (ERO), nitrogênio (ERN) e cloro (ERC) normalmente associadas a doenças degenerativas. Recentemente, diversos esforços têm sido realizados no uso de fontes naturais de baixo custo para produzir novos produtos de valor agregado e isso pode ser feito usando resíduos de indústrias de uva para extrair antocianinas e compostos antioxidantes que ajudam a desenvolver novos usos para materiais descartados e promover sustentabilidade.

Atualmente, a alta pureza de polifenóis é importante para a área farmacêutica e dos alimentos funcionais. Métodos convencionais de extração, como maceração e Soxhlet, mostraram baixa eficiência e potencial poluição ambiental devido a grandes volumes de solventes orgânicos e longo tempo de extração necessário (Dai and Mumper 2010). Os principais solventes orgânicos usados são metanol, etanol, acetona, acetato de etila e suas combinações (Xu and Chang 2007). Vários métodos foram desenvolvidos, como microondas, extrações assistidas por ultra-som, extração subcrítica de água (ESA), extração com fluido supercrítico (EFS), extração com fluido pressurizado (EFP) ou extração com solvente acelerado (ECSA). (Dai and Mumper 2010) Esses métodos têm algumas desvantagens, como longos ciclos de produção e alto custo (Fu, Zu et al. 2006, Farías-Campomanes, Rostagno et al. 2013) .

Portanto, é importante estudar métodos ecológicos simples e eficientes para extrair antocianinas. Com as tendências da química verde, resinas macroporosas MARs são materiais poliméricos porosas desenvolvidos com excelentes propriedades adsorventes devido a suas estabilidades físicas e químicas, grandes áreas superficiais, fácil regeneração, longa vida útil (Chen, Zhang et al. 2010), rápida taxa de adsorção, forte capacidade de adsorção, fácil eluição e alta seletividade (Wan, Sheng et al. 2014). Adicionalmente, as MARs possuem alta resistência mecânica, boa resistência ácida e alcalina, muitos grupos funcionais, que conferem diferentes polaridades (Liu, Liu et al. 2010).

A separação é baseada nas diferenças de peso molecular, grupo funcional, polaridade e forma das diferentes moléculas na solução, por isso elas mostram diferença de afinidade entre os diferentes materiais adsorventes. Desta forma, a adsorção é bastante seletiva e permite a recuperação de constituintes a partir de soluções aquosas diluídas, bem como de sistemas não aquosos através de força eletrostática, interação de pontes de hidrogênio, complexação e ação de peneiramento de tamanho, etc. (Liu, Zhang et al. 2010). Muitos fitoquímicos de origem vegetal, como genisteína e apigenina de extratos de raízes de guandu, licopeno de extratos de casca de tomate, rosavina de Rhodiola rosea são investigados para purificação por adsorção (Ma, Tao et al. 2009, Gao, Yu et al. 2013).

Até agora, as resinas macroporosas têm sido usadas com sucesso na separação e enriquecimento de fitoquímicos, como saponinas, (Jia and Lu 2008) taxol (Fu, Zu et al. 2008) isoflavona (Liu, Zhang et al. 2010), antocianinas (Chang, Wang et al. 2012) e levana (Liu, Luo et al. 2010) de fontes naturais. As resinas Amberlite XAD-7 e XAD-16 também têm sido amplamente

usadas na purificação de antocianinas (Sadilova, Stintzing et al. 2006, de Rosso, Hillebrand et al. 2008, Sun, Cao et al. 2010) purificando as antocianinas dos extratos de frutos de framboesa vermelha utilizando sucessivamente cromatografia em coluna Amberlite XAD-7, filtração em gel Sephadex LH-20 e HPLC preparativa, no entanto, as resinas adsorventes Amberlite XAD são caras para a purificação de antocianinas. Portanto, é importante encontrar uma resina macroporosa de baixo custo e altamente eficiente para purificar as antocianinas de resíduo da fermentação de casca da uva *Alicante Bouschet* (RFCU). Apesar de existam estudos sobre a extração de compostos fenólicos de bagaço de uva, nenhum estudou a separação de compostos de antocianinas do resíduo da fermentação de casca da uva (RFCU) (*Alicante Bouschet*) utilizando resinas macroporosas.

OBJETIVOS

Objetivo geral

Separar e purificar de compostos de antocianina a partir de do resíduo da fermentação de casca da uva RFCU usando resinas macroporosas.

Objetivos específicos

- Extrair de compostos de antocianina usando soluções ambientalmente correto.
- Avaliar a concentração e os tipos de compostos de antocianina presentes no bagaço de uva (*Alicante Bouschet*).
- Análisar do perfil de antocianinas em extratos de bagaço de uva antes e após processos de adsorção e dessorção.
- Identificar características de operação de adsorção e dessorção (ensaios estáticos, cinéticos e isotérmicos) para resinas macroporosas.

CAPÍTULO I: PROPERTIES AND BENEFITS OF ANTHOCYANINS: A REVIEW

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Properties and benefits of anthocyanins: a review

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Abstract

Anthocyanins are spread in different kinds of food include especially fruits and floral tissues with extensive range of anthocyanin compounds reach more than 500 exist in nature. Anthocyanins can be used as quality-giving additives in food industries due to its characteristics as product-enhancing colorants and consequently a plenty of studies about sources of anthocyanins and various methods to extract them were reported. Many studies about chemical properties and stability of anthocyanin have been done, as well as a little of medical studies about effect anthocyanin to inhibit some intractable diseases were researched. However there are lacks of researches about health benefits of anthocyanins that derived from its properties as antioxidants and health promoting materials. In this paper an attempt to shed light on many studies to present some important properties of anthocyanin, antioxidant effect, health and nutritional benefits. Eventually gain a clear view of the future importance of anthocyanin and possibility to use it as health-saving therapeutic and prophylactic applications.

Keywords: polyphenols, antioxidant, pigments, pharmaceutical.

Introduction

Polyphenols are aromatic organic compounds consisting of one or more phenyl rings associated with a hydroxyl molecule; these substances are naturally found as secondary plant material(Morello, Shahidi et al. 2002). These polyphenols in the plant have color, taste and smell, in addition to protect the plant from its enemies (i.e. insects, fungi, etc.) or attract beneficial insects, especially bees for transporting pollen and enrichment. Polyphenols protect the plant against oxidative effects, acting as antioxidants; and keep them safe from harmful ultra violet (UV) rays during photosynthesis. Anthocyanins are considered as glycosidically bound anthocyanidins available in many fruits and flowers. While flavones and chalcones are yellow, anthocyanins are pigments responsible for the violet, bright red and blue colors of fruits and other foods (Mazza 2018). Anthocyanin compounds play an important role in food particularly fruits in addition to floral tissues (Harborne and Williams 2001, Ai, Naing et al. 2017). Anthocyanins are also used as nutrients in many kinds of food in the form of powder extracted from fruits, These compounds can be extracted and used in food industries (Bridle and Timberlake 1997, Mazza 2018).

The unique properties of anthocyanins as antioxidants and their potential benefits to human health have been demanded by researchers in order to reach the effective applications in medicine and nutrition areas(Azeredo 2009).

Recently, many studies were implemented about phenolic compounds and anthocyanins due to their important role for health and food quality. These phytochemicals are associated with counteracting the risk of cardiovascular diseases, cancer and cataract as well as a great number of other degenerative diseases (Singleton 1981, Hollman 2001, Su, Wang et al. 2018), resulting with their effective role in reducing oxidation processes of lipids, protein cross linking and DNA mutation and, at later stages, tissue damage. Therefore, the interesting in aorta atherosclerotic plaque dose-dependently and anthocyanin consumption has been increased due to good effects of anthocyanins in this regards.

The importance of anthocyanin for the human health has been reviewed in many studies, (Long, Zhang et al. 2018) which refer to remarkable anti-tumor capacity of mulberry anthocyanins (MA) in repressed thyroid cancer cells where SW1736 and HTh-7 cell proliferation were suppressed by MA in a time- and dose-dependent manner. In poinsettia leaves where anthocyanin biosynthesis is accompanied by thylakoid membrane unstacking and loss of photosystem II complexes that work as a tool to avoid reactive oxygen species (ROS) formation (Moustaka, Panteris et al. 2018). Anthocyanins can be also helpful against development of ischemic heart diseases because of their ability to limit oxidative stress (Miguel 2011, Kruger, Davies et al. 2014) and remove ROS by various mechanisms like direct scavenging of ROS- reactive oxygen species (Miguel 2011) and induction of enzymes responsible for ROS removal (Kruger, Davies et al. 2014). Many diseases are occurred by high cholesterol which can be lowered by anthocyanin, (Wang, Zhu et al. 2018) reported that a dose-dependent increase in excretion of both neutral and acidic sterols accompanied by decrease on plasma total cholesterol and diets.

The objective of this paper is to review some specific properties of anthocyanin, health benefits and their importance in food. In addition to present more information about anthocyanin compounds, and their large diversity.

Anthocyanin properties

Chemical properties

The anthocyanin is mainly formed of anthocyanidin molecule, according to the (**Figure.1.**) appear the anthocyanidin (aglycons) is constructed of an aromatic ring (A) linked to an heterocyclic ring that has oxygen atom in its structure that is also linked by carbon-carbon connection to the final aromatic ring (B) (Konczak and Zhang 2004) .In this formation of anthocyanidin with a sugar moiety it is called as anthocyanin.

A large diversity of anthocyanins exists in the natural world. The number of hydroxylated groups is the main reason to their variety (**Figure.2**.), the number and kind of the sugars molecules that are linked to their structure, the aromatic and aliphatic carboxylates linked with the sugar in the molecule and the location of these bonds (Kong, Chia et al. 2003). Till now the reports indicate to more than 490 different kind of anthocyanins (Al-Fartosy and Abdulwahid 2015) and 23 of anthocyanidins that contain only six of the most spread in vascular plants (Clifford 2000).

In protic solvents the synthetic flavylium salts FVs exhibit red coloring, whilst in aprotic solvents the solutions are yellow (Ito, Tanaka et al. 2002). This fact has been explained by proposing that the red and yellow species correspond to a monomer and dimer respectively; therefore, when increasing FVs concentration, the red coloring is favored.
pH has a strong effect on the chemical form of anthocyanin (**Figure.1**) (da Costa, Nelson et al. 1998), for example at pH 1, the *flavulium* cation is the dominate kind and give the red and purple colors (**Figure.1A**). Furthermore, higher values of pH more degradation of anthocyanin occurred due to a potential changing in the structure of anthocyanin with different level of pH where (Jiang, Mao et al. 2019) found that at higher level of pH (pH 5.0 and pH 7.0). The color gradually turned to brown with absence returning to red color which can be caused by complete degradation of anthocyanins in the neutral environment.

Where the pigments and colorless organic components, or metallic ion, shape complicated associations, coming with an increment or change in the color intensity, thus phenomenon is called co-pigmentation (Boulton 2001) like the co-pigmentation of anthocyanin with phenolic co-pigments in black chokeberry (Klisurova, Petrova et al. 2019). The interaction of anthocyanin-co-pigment can be happened by five of different ways based on the interacting species (**Figure.3**). When another compound of anthocyanin is the co-pigment, an intermolecular or a self-association is created (**Figure.3A and B**); if the interaction take place with a metal , a complexation is happened (**Figure.3C**); when the co-pigments is with free electron pairs, an intermolecular co-pigmentation is carried out (**Figure.3D**); at the end, in the highest complex case, the co-pigmentation can be happened at the same time by sugar, aglycon, protons and co-pigment.

Many types of anthocyanins have been extracted from roses, flowers, leaves and fruits. The most species of anthcyanidins discovered in nature are cyanidin, malvidin, pelargonidin, peonidin, petunidin and delphinidin (**Figure.4**.). 3,5- diglusosides and Anthocyanidin 3-glucosides are the most quite prevalence anthocyanins (Goodwin 1965, Zhang, Chen et al. 2019) . In the media of high acidic level (pH<2.5) anthocyanins give stabilized flavylium ions with red or orange color (**Figure.5.**). In the acidic media of anthocyanin that reach to levels of pH 4 to 6, the color of violet anhydrobase is created first and it decolorizes fastly (**Figure.4.**) to give a pseudobase due to hydration process (Brouillard and Delaporte 1977, Brouillard 1982, Cheminat and Brouillard 1986, Rakić, Rinnan et al. 2019). Because of the weakly acidic the flower cell sap, anthocyanins cannot be stabilized in the cell sap in the case of absence additional stabilizing factors.

The co-pigmentation anthocyanins are affected by bathochromic as stabilizing factor. In the polyphenols such as tannins and flavones the hydrogen bonding between co-pigment used and the host compound are attributed as co-pigmentation effects. The co-pigmentation can be happened between phenolic acids and anthocyanins in wine residues resulting with enhance the bathochromic-shift, color, thermal, light and oxidation stability of wine residues anthocyanins WRA solutions, these co-pigmentations take place easily via the non-covalent interactions, e.g., hydrogen bonding, π-π stacking, and van der Waals interaction, due to their flexible chemical structures. . Thus, the structural characteristics of co-pigments were mainly related to the co-pigmentation effects of anthocyanins (Fan, Wang et al. 2019) (Chen and Hrazdina 1981).

However, based on phenomenon called glycosylation and acylation anthocyanin **(Figure.6.),** it can reach to properties more resistance to light and temperature with less degradation of anthocyanin pigments and more stability due to forming an intermolecular H-bonding network within the anthocyanin molecule (Borkowski, Szymusiak et al. 2005). There are different kinds of acylating groups can be attached to glycosylated aglycones with two types of acids (cinnamic and/or aliphatic) are used to acylate anthocyanin. (Moloney, Robbins et al. 2018) reported that hydroxycinnamic acid HCA residues promote π-stacking interactions between the phenolic nuclei in mono- and diacylated anthocyanins of red cabbage, therefore efficiently protecting the cyanidin chromophore against water addition leading to colorless forms besides to improve resistance against the long-term color loss in mildly alkaline conditions.

Anthocyanin in various different kinds of foods:

Because they show health-promoting effects such as lowering incidences of cancer and reducing blood pressure and cardiovascular diseases, vegetables and fruits are excellent sources of anthocyanin (Cook and Samman 1996, Krga and Milenkovic 2019) (Table.1.). Furthermore, anthocyanins and related enzymes influence the quality of vegetables and fruits (Cantos, Espin et al. 2001).

Bilberry (*Vaccinium myrtillus*) is one of the fruit that is rich in phenolics include flavonoids and phenolic acids (Colak, Primetta et al. 2017); the bilberry has been used in producing of medicine due to its pharmacological features (Colak, Primetta et al. 2017) (Azar, Verette et al. 1987), The reports indicate to the rate of total anthocyanin content range from 3,700 to 6,980 mg/kg of fresh weight (Nyman and Kumpulainen 2001) and from 22,980 to 30,900 mg/kg of dry weight (Kähkönen, Hopia et al. 2001). Three anthocyanins are found in bilberry fruit powder (**Figure. 7**.) (Chandra, Rana et al. 2001). This content of anthocyanin is lower in the processed bilberry products where the highest content of total anthocyanins was determined in fresh fruits (5,190 mg/100g DW) whereas the lowest content of total anthocyanins (921 and 1,099 mg/100 g DW) was determined in fresh and pasteurized juices (Zorenc, Veberic et al. 2018).

The total content of phenolic compounds in *Georgia-grown* blackberries is 4,865.3 mg/kg of fresh weight expressed as gallic acid equivalents (Sellappan and Akoh 2002). Furthermore, detected two acylated anthocyanins in unripe blackberries and reported that their levels fastly lowering while the ripening process. Later, (Fan-Chiang 1999) discovered cyanidin-3-glucoside acylated with malonic acid in blackberry while (Stintzing, Stintzing et al. 2002) isolated cyanidin dioxalylglucoside-a novel zwitterionic anthocyanin from evergreen blackberry (*Rubus lacitianus Wild*.). Of these, cyanidin-3-glucoside is considering the major anthocyanin in blackberry (Garcıia-Viguera, Zafrilla et al. 1997). (da Silva, Itoda et al. 2018) looked into availability of phenolic compounds in Brazilian cultivar blackberries (cv. Xavante) after processing into purées where anthocyanin presented stability in mild heat

treatment (70 ^{0}C for 20 min), however anthocyanin was degraded at intensive heat treatment of purée (80 ^{0}C for 20 min).

Blackcurrants are widely used for production of jams and juices; yogurts are also prepared by this are cyanidin 3-rutinoside, delphinidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3- glucoside. These four main anthocyanins make up more than 97% of total anthocyanins kind of fruit. Blackcurrant berries is considering as a rich source of phenolic compounds such as flavonoids, anthocyanins proanthocyanidins and phenolic acids (Costantino, Albasini et al. 1992, Häkkinen and Auriola 1998, Tabart, Auger et al. 2018). Blackcurrant anthocyanins are found in the skin of berries (Iversen 1999); their total content ranges from 1560 to 10,640 mg/kg expressed as cyanidin 3- glucoside equivalents (Moyer, Hummer et al. 2002). The predominant anthocyanins of blackcurrants in blackcurrants (Slimestad and Solheim 2002). Other anthocyanins detected in blackcurrants include: Cyanidin-3-(6″-coumarylglucoside), Cyanidin-3-arabinoside, Malvidin-3-rutinoside, Malvidin-3-glucoside, Peonidin-3-rutinoside, Peonidin-3-glucoside, Delphinidin-3-sophoroside, Cyanidin-3-sophoroside, Pelargonidin-3-rutinoside, Delphinidin-3-(6″-coumarylglucoside) (Slimestad and Solheim 2002).

(Mattila, Hellström et al. 2011) studied the anthocyanin content of some European commercial blackcurrant juice products that were 4.3 (brand UK1) and 58 mg/2.5 dl (brand G1) expressed on a ready-to-drink beverage basis, the mean of anthocyanin content varied widely between Finnish, Polish, German, and British products (12, 32, 38, and 7.5 mg/2.5 dl, respectively, $p < 0.0001$).

Highbush (cultivated) blueberries and *Lowbush* (*wild*) have been used for many years to produce berries on a commercial scale (Kalt, Ryan et al. 2001). Blueberries are a rich source of anthocyanins (Sellappan, Akoh et al. 2002, Wu, Han et al. 2018) . Blueberry phenolic compounds show inhibitory effects against chemically induced (Sellappan, Akoh et al. 2002). The content of anthocyanin in blueberry is affected by the genetic differences (cultivar), degree of maturity at harvest, processing, preharvest environmental conditions and postharvest storage conditions. The content of anthocyanin in various cultivars of highbush and half-high blueberries has been studied by (Li, Li et al. 2017), malvidin 3-galactoside was the major anthocyanin (17.8 ± 6.9%), followed by malvidin 3-glucoside (13.7 ± 5.1%) and delphinidin 3-galactoside (13.2 ± 2.4%), and, together, they accounted for more than 45% of the total anthocyanins present in all cultivars and their average concentrations were 32.8 ± 18.9, 24.2 ± 13.4, and 24.4 ±11.5 mg 100 g^{-1} FW, respectively. (Ehlenfeldt and Prior 2001) surveyed anthocyanins and the total content of phenolics in 87 highbush blueberry (*Vaccinium corymbosum L.*) and species-introgressed *highbush* blueberry cultivars. The total anthocyanin content was between 890 and 3310 (1790 on average) mg/kg of fresh weight expressed as cyanidin-3-glucoside equivalents.

The fruits of European cranberries, American cranberries, *Vaccinium macrocarpon* and *Vaccinium oxycoccus* possess a bright red color and a distinctive flavor; they are sold processed into sauce or fresh, concentrates and juice. Cranberry fruits serve as an excellent source of anthocyanins (Prior, Lazarus et al. 2001, Wang, Li et al. 2015).

The predominant anthocyanins in American cranberries are 3-O-arabinosides and 3-O-galactosides of peonidin and cyanidin, while European cranberries contain 3-Oglucosides of cyanidin and peonidin . Posteriorly, (Prior, Lazarus et al. 2001) reported the presence of small quantities of cyanidin 3-O-glucoside and petunidin 3-O-galactoside in American cranberry fruits. The total content of anthocyanins in cranberry fruits ranges from 180 to 656 mg/kg of fresh weight (Wang and Stretch 2001, Cătunescu, Rotar et al. 2019); these are located under the fruit . Cyanidins comprise approximately 55% of total anthocyanins in cranberry (Prior, Lazarus et al. 2001). The content of anthocyanins is affected by postharvest conditions, cultivar and fruit size (Wang and Stretch 2001). Smaller berries contain higher levels of

anthocyanins than those of larger ones due to the location of anthocyanins in the fruits (Wang and Stretch 2001). Storing cranberries for 3 months at temperatures of up to 15°C significantly increases their total anthocyanin content (Table 2); however, this increase is somewhat lower when cranberry fruits are stored at the recommended storage temperature of 2 to 4°C (Wang and Stretch 2001).

Anthocyanin showed good stability during dried powder preparation of cranberry (*Vaccinium macrocarpon L.*) in comparing with other phenolic compounds(Cătunescu, Rotar et al. 2019), (Oszmiański, Kolniak-Ostek et al. 2015) indicated 5.6-fold higher concentration of anthocyanins in pomaces compared with juices with quantitatively dominant were the myricetin derivatives, then quercetin and methoxy quercetin.

Punica granatum (Pomegranate) is considered as one of the oldest recognized edible fruits and is grown in India, Japan, U.S., Afghanistan, China, Iran, Mediterranean countries and Russia. It is sold raw or processed to sauce and juice. The soluble polyphenols content varies from 0.2 to 1.0 g/100 g (Rinaldi, Caligiani et al. 2013), being anthocyanins one of the most important together with lignans gallagyl-type tannins, ellagic acid derivatives, and other hydrolysable tannins which contribute to the antioxidant activity of the juice (Bonzanini, Bruni et al. 2009).

(Gil, Tomás-Barberán et al. 2000) reported that cyanidin 3-glucoside (59.5 to 128.3 mg/L) is the major anthocyanin in pomegranate juice. Recently (Legua, Forner-Giner et al. 2016) showed that cyanidin 3-O-diglucoside is the predominant anthocyanin in pomegranate juice, in addition to other anthocyanins were identified including cyanidin 3,5- . O-diglucoside, pelargonidin 3-O-diglucoside, delphinidin 3-O-diglucoside, delphinidin 3,5-Odigluside and cyanidin pentoside.

Red onions contain a number of anthocyanins that are particularly concentrated in the outer fleshy layerand and skin (Gennaro, Leonardi et al. 2002, ZHANG, Peng et al. 2016). (Herrmann 1976, ZHANG, Peng et al. 2016) identified delphinidin 3,5-diglycosides, cyanidin 3-glucoside, cyanidin 3,5-diglycosides and peonidin 3-glucoside in red onions. Subsequently, (Gennaro, Leonardi et al. 2002) found petunidin and delphinidin derivatives in red onions. According to (FOSSEN, ANDERSEN et al. 1996, Donner, Gao et al. 1997) cyanidin 3-glucoside, cyanidin 3-(6″-malonylglucoside), and cyaniding 3-(6″-malonylglucoside-3″-glucosylglucoside) include over 95% of total anthocyanins in whole red onions. On the other hand, (Gennaro, Leonardi et al. 2002) have explained that delphinidin derivatives constitute about 30% of total anthocyanins and cyanidin derivatives comprise over 50% of total anthocyanins in whole red onions.

The different red-fleshed potato cultivars (*Solarium stenotum* and *Solanum tuberosum*) contain monomeric anthocyanins with the total content ranges from 24 to 403 mg/kg of tuber (Rodriguez-Saona et al., 1998). Higher levels of anthocyanins have been detected in the skin of purple potato cv. Urenika (5078 mg/kg) than in the flesh (1836 mg/kg) (Lewis 1996). Otherwise, a higher concentration of anthocyanin has been discovered in the flesh than in the skin of red-fleshed potatoes. The main anthocyanins in purple-fleshed potatoes were identified as acylated anthocyanins petunidin-coumaroylrutinoside-glucoside and peonidin-p-coumaroylrutinoside glucosidev (Heinonen, Farahmandazad et al. 2016).

Chlorogenic acids and anthocyanins are the major phenolic compounds detected in sweet potato tubers (Suda, Oki et al. 2002).The purple-fleshed sweet potato tuber is a good source of anthocyanins; the main anthocyanin pigments in sweet potato include mono- and diacetylated forms of peonidin and cyaniding. (Suda, Oki et al. 2002) reveals the chemical structures of some anthocyanins discovered in purple-fleshed sweet potato. The total content of anthocyanins in sweet potato is 590 mg of peonidin 3-caffeoylsophoroside-5-glucoside equivalent/kg (Furuta, Suda et al. 1998). Acylated cyaniding and acylated peonidin constitute

19 and 74% of anthocyanin pigments in sweet potato *cv. Ayamurasaki*, respectively (Suda, Oki et al. 2002).

Antioxidant properties of anthocyanins:

In addition to the coloring effects in fruits, anthocyanins is known with his ability to inhibit the oxidation of lipid in different medias such as human low-density lipoprotein (LDL) in liposome and vitro (Satué-Gracia, Heinonen et al. 1997, Svanberg, Malmberg et al. 2019) and scavenging activity contra diverse artificially produced free radicals (Vinson, Dabbagh et al. 1995, Wang, Cao et al. 1997) .

In the foods and biological systems, antioxidant examinations can be identified in two groups: the first that measure free radical scavenging ability and the second that evaluate lipid peroxidation (Miguel 2010). The antioxidant activity in the assessing lipid peroxidation can be detected by measuring the substrate and the oxidant consumption, and the intermediates or the final products formation, and several lipid substrates can be used. There are methods to measure free radical scavenging ability which can be divided in two groups according to the chemical activities involved: hydrogen transfer-based tests and transfer of electron.

Furthermore, the efficiency to scavenge various reactive oxygen species (ROS) vary from one anthocyanin to another, for example, delphinidine is considered the highest active contra the superoxide anion (followed by pelargonidin and cyanidin) and pelargonidin is considered the most effective against the hydroxyl radical (Antal, Garban et al. 2003). In general, the pyrone ring with the number of free hydroxyls around play a significant role in antioxidant activity of anthocyanins. More number of hydroxyls more antioxidant activity, stable anthocyanin-metal compositions can be generated quickly by anthocyanins with their 3',4'-dihydroxy groups that chelate metal ions (**Figure.8.**) (Sarma, Sreelakshmi et al. 1997). Anthocyanins at pH 2-4 especially available in the form of flavylium cations and due to the charge allocation this make them liable to attack of nucleophilic compounds on points of 2 and 4. Regarding to (Tsuda, Shiga et al. 1996, Antal, Garban et al. 2003), it can be assumed that the process of hydroxylation at these points improves chelating capacity of anthocyanin, for example, ascorbic acid from metal-motivated oxidation (**Figure. 8.**).

Anthocyanins turn to be more difficult to absorb especially when it bond with sugars. In order to get satisfying absorption by the gut they must be hydrolyzed to phenolic acids or anthocyanin aglycones. Despite these results, it was reported that under simulated gastrointestinal conditions, the bulk of anthocyanins were unsteady in the condition of small intestine and hypothesized that their biological activities were executed by unidentified breakdown products.

Extraction properties:

The tend to employ anthocyanin as additive materials in food industries to enhance quality and sensory properties of food or as medical compounds in the field of pharmaceutical industries make the scientific research to optimize extraction of anthocyanin occupy an intense interesting to implement this task. As blueberry is a rich source of anthocyanin (Grace, Ribnicky et al. 2009), the extraction process of it with high yield, premium quality and high efficiency is an important topic for researchers and industrialists. Microwave assisted extraction (MAE) is a feasible method for the extraction of anthocyanin from blueberries (Zheng, Xu et al. 2013). Fast extraction and effective separation are the typical advantages of MAE technology. These properties are derived from the disruption of cell structure happened by the penetrated heating from microwave irradiation (Zhongdong, Guohua et al. 2006, Gao, Huang et al. 2007). Microwave assisted extraction of plant

secondary metabolites can be affected by many diversities of factors, such as frequency and power of microwave, moisture content and particle size of plant samples, duration of microwave radiation, concentration and type of solvent, extraction temperature ,ratio of solid to liquid,, extraction pressure and number of extraction cycles (Wang and Weller 2006). Of these parameters, solvent is considered as one of the most important factors for microwave assisted extraction, which can affects the absorption of microwave energy determined by its dissipation factor and solubility of the target components (Chen, Jin et al. 2008), in this contest, (Yang and Zhai 2010) reported that extraction efficiency of total anthocyanin content using microwave-assisted extraction was better than conventional extraction.

Various factors as type of solvents, temperature and pH can effect on the anthocyanin extraction from organisms, the solid-to-liquid ratio has the most important effect on the anthocyanin extraction kinetic from powdered blueberry, followed by the ethanol concentration and extraction temperature, and the last is the extraction time (Zheng, Xu et al. 2013) where the highest anthocyanin extraction rate of 74.31% was obtained with high reliability under the solid-to-liquid ratio of 1:30. (Dai and Mumper 2010) explained that ethanol plays a major role to rupture the hydrogen bonds and hydrophobic bonds existing between anthocyanins– proteins and anthocyanins–cellulose in the water–ethanol system.

The solubility of material depends on the intermolecular or interionic forces of solute and solvent. The molecules of solvent have sufficient attraction to break up the hydrogen bonds between molecules or the dispersion forces between molecules in the solvent for the solute particles (Abyari, Heidari et al. 2006). Increasing solid to liquid ratio enhances the concentration gradients, which promotes the diffusion of anthocyanin within the material to elevate the anthocyanin extraction rate. With the further increase of solid-to-liquid ratio, the activity of certain polar phenolics (other than anthocyanin) within blueberry particles were improved to diffuse, which weakened the dissolution of the anthocyanin in ethanol (Türker and Erdogˇdu 2006). In conventional extraction, such as pressurized liquid extraction or heat reflux extraction, the positive impact of solvent-to-solid ratio was found on the enhancement of target component yields (Chemat, Aït-Amar et al. 2005).

Extraction anthocyanin from purple (Yang, Chen et al. 2009) with 1 M HCl–95% ethanol (15:85, v⁄v) found that the anthocyanin yield increased significantly with the temperature from 10 to 50 Cº, but it did not increase any further above 50 ˚C. This suggested that the optimal temperature for anthocyanin extraction was 50 ºC. (Türker and Erdogˇdu 2006) also suggested that as the temperature raised (25–50 ˚C), the anthocyanin yield increased. However, higher temperature may also result in degradation of anthocyanin as reported by (Cacace and Mazza 2003).

Another study (Fan, Han et al. 2008) looked into the effect of temperature, time and ratio solid : liquid on extract of anthocyanin from purple sweet potato and found that the highest yield (158 mg/100 g of dry weight dw) of anthocyanin were reached at the temperature 80 ºC, extraction time 60 min, and solid–liquid ratio 1:32. However the low temperature increased the oxygen solubilization. In this moment, the oxygen presence could cause browning reactions, increasing the musts hue and the brown color. Concurrently, the red color could be decreased because the anthocyanins could be substrates of different reactions. In this regard, it is demonstrated that anthocyanins are substrates in the browning reactions, but they can also participate in several direct condensation reactions with themselves or with tannins, in condensation reactions with flavanols, through or not a bridge methylmethyne (Pissarra, Lourenço et al. 2004, Marquez, Serratosa et al. 2013) and/or co-pigmentation reactions.

Benefits of anthocyanin:
Anthocyanin as colored materials in food:

Anthocyanins involve a group of naturally occurring pigments that give various colors of most species in the plant kingdom like violet blue, red, purple, and magenta coloration (Harborne and Williams 2001). These polyphenolic compounds are polyhydroxy, glycosides of anthocyanins, and polymethoxy derivatives of flavylium salts or 2-phenylbenzopyrylium (**Figure 9**).

A big number of acyl groups and glycosyl which may bind with sixteen different naturally occurring anthocyanidins led to more than 400 different anthocyanin pigments which have been highlighted in the previous studies of literature (Kong, Chia et al. 2003). The highly reactive and amphoteric nature of these pigments, in addition to their structural diversity, has made their quantitation and identification tiresome and difficult.

There are different applications of anthocyanin extracts comprise preserves, coloration of acid fruit preparations and jams. However, the use of extracts in these applications is mainly depending on the quality and nature of the fruit (sulphited, fresh and frozen) and availability of proteins. For example, extracts containing oliogomeric pigments or other phenolic compounds higher than a specific level cannot be used to color jellies and tend to form precipitates with gelatin (Bridle and Timberlake 1997). In confectionary sugar, grape extract (0.4% w/w) generate a clear color of ruby red in the boiled sweets, which can be modulated by colorants of other fruit sources. (Giusti and Wrolstad 2003) looked into the ability of acylated anthocyanins from extracts of grape skin, red radish, red cabbage and black carrot to color the products of dairy like sour cream and yogurt which have pH levels around 4.2 - 4.5. They discovered that carrot and radish alone or together could give an eligible color of red hue for dairy at concentrations lower than 5 mg monomeric anthocyanin/100 g sample. Because of the short shelf-life of these tested dairy products that are only a few weeks under refrigeration, the steadiness of anthocyanin extracts would not be influenced making them viable alternatives. With rising value of pH, discoloration happens, however if the product that being colored comprise components eligible of acting as co-pigments, in this case color may be retained and also light-stabilized to a certain extent (Bridle and Timberlake 1997). Therefore, the use of acylated anthocyanins with enhanced color and stability to heat, good condition of light and pH could achieve a promise to make anthocyanins as natural food colors. In the future, It is predicated that the production and adding of anthocyanins as natural food colorants will constantly increase, following the current tendency away from synthetic colors (Horbowicz, Kosson et al. 2008).

Anthocyanin as therapeutic and prophylactic compounds:

Anticancer properties:

Anticancer properties of anthocyanin have been reported in many studies indicating a positive relation between cancer inhibition and the intake of anthocyanins-rich food (Bunea, Rugină et al. 2013, Filipiak, Hidalgo et al. 2014, Forester, Choy et al. 2014), the effect of anthocyanins to inhibit a cancer can be in different ways including preventive and therapeutic effects, however the mechanism of anthocyanins as anticancer compounds need to be more evidenced in the future. Here we present some experimental results associated with anticancer properties of anthocyanins.

The anticancer activities of anthocyanin from different kinds of food especially fruits have been reported to inhibit the initiation, and progression of several cancers like cervical cancer (Rugină, Sconţa et al. 2012), blood cancer (Tsai, Huang et al. 2014), lung cancer (Aqil,

Gupta et al. 2012), breast cancer (Devi, Kumar et al. 2011), liver cancer (Bishayee, Háznagy-Radnai et al. 2010) and prostate cancer (Reddivari, Vanamala et al. 2007).

Different mechanisms have been demonstrated about the work of anthocyanins as anticancer compounds, (Long, Zhang et al. 2018) showed that mulberry anthocyanins MA repressed HTh-7 and SW1736 cell proliferation in a dose- and time-dependent method, most important, it was voided the anthocyanin prompted cell death by 3-methyladenine (3-MA) or chloroquine diphosphate salt (CQ) treatment, supposing that MA-induced HTh-7 and SW1736 cell death was partially dependent on autophagy. Based on Differentiation induction, anthocyanin can induce differentiation ending of tumor cells and discontinue tumorigenesis, (Fimognari, Berti et al. 2004) resulted that cyanidin-3-O-β-glucopyranoside could induce the differentiation of human sharp promyelocytic leukaemia cell line HL-60 in a dose-dependent manner by activating protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K) . In regard to Cellular transformation which is one of the mechanisms underlying tumorigenesis (Limtrakul, Yodkeeree et al. 2015) reported that black rice whole grain extracts might repress LPS-motivated inflammation via suppression of the MAPK signaling pathway, causing decreased NF-κB and AP-1 translocation. (Anwar, Fratantonio et al. 2016) reported that a standardized berry anthocyanin rich extract prevented the proliferation of Caco 2 cells by upregulating the expression of p21Waf/Cif1, arresting their cell cycle, and moreover inducing them to subject apoptosis by Caspase-3 activation.

Anti-Diabetic and Anti-fatness Properties:

It is proposed that a diet rich in fruits and vegetables, particularly rich in phenolics and low in fat may decrease the hazard of type-2 diabetes and fatness which is a case related with resistance of insulin. Insulin resistance is a defect in which insulin insufficiently activates glucose transport in fat and inadequately inhibit hepatic glucose production (Ghosh and Konishi 2007). Till now it is unclear which reasons and mechanisms prohibit adequate amounts of insulin to be excreted from the beta cells of the pancreas. Oral hypoglycemic agents such as sulfonylurea- based drugs, which directly stimulate insulin release from beta cells; these agents are actually being used to prevent insulin resistance and help regularize the levels of blood glucose. However, such as most drugs they have disadvantages such as weight earn and deficiency of ability to organize normal levels of blood glucose (Ghosh and Konishi 2007). For this, it is considered more healthy and affordable to control levels of blood glucose or increase insulin production by ingesting a diet rich in polyphenol like fruits and vegetables. (Rojo, Ribnicky et al. 2012) found oral-taking ANC enhanced glucose tolerance and fasting blood glucose levels in hyperglycaemic obese C57BL/6J mice that fed a high fat diet. In H4IIE rat liver cells, ANC reduced glucose production and improved the insulin-stimulated down regulation of the glucose- 6-phosphatase gluconeogenic enzyme, likewise (Takikawa, Inoue et al. 2010) reported that anthocyanin enriched fractions of bilberry produced hypoglycemic and insulin sensitizing effects in type II diabetic mice.
Anthocyanin can work to increase lipid metabolism in the body resulted in reducing body weight , this levels of bioactivity are different among various kinds of anthocyanins compounds where (Skates, Overall et al. 2018) reported that lipid-decreasing efficiency in mice was in the order of Malvidin> delphinidin > Cyanidin.

With regard to glucose-lipid metabolism and level of inflammatory cytokines, anthocyanin can improve the both leading to alleviate metabolic syndrome and obesity where **(Wei, Zhang et al. 2016)** indicated that aornia melanocarpa anthocyanin (AMA) restricted liver injury and obesity induced hepatic steatosis. Moreover, AMA obviously enhanced inflammatory cytokines including both IL-6 and TNF-α in liver and fatty tissue.

Properties of Oxidative Stress and Lipid decreasing:

The major factor of the insulin resistance syndrome is increased level of triglyceride (hypertriglyceridemia) (Ghosh and Konishi 2007). Various studies have established a relation between a diet that is high in fat with the evolution of type-2-diabetes and to induce hyperlipidemia, hyperinsulinemia and hyperglycemia. Recently, (DeFuria, Bennett et al. 2009) demonstrated that blueberries can work against insulin resistance in whole-body by preventing the early inflammatory events in tissue of adipose and can also enhance glycemia in an animal model. All of the insulin-resistance associated marks (increased oxidative stress and up-regulation of inflammatory genes) that were noticed in the mice fed on a diet contain high levels of fat were not exist in the mice fed on supplemented diet of blueberry powder (contain 31.44 g/kg dry weight of anthocyanins). Moreover, a lowering in hyperglycemia was discovered in rats fed on blueberry powder coincident with decreasing in adipocyte death. In a similar way, (Tsuda, Horio et al. 2003) found that anthocyanins (cyanidin-3-glucoside) from purple corn (2 g/kg diet) significantly inhibited the increase of obesity and reduced hyperglycemia resulted by a high fat diet in mice.

Additionally, a remarkable lowering in serum free radical levels was found in patients of high blood oxidative stress state. Similarly, they were demonstrated a beneficial oxidative effects in a clinical study by (Rosenblat, Hayek et al. 2006) following consuming of 50 mL of pomegranate juice for 3 months. Pomegranate Juice (384 mg/L anthocyanins) that was consumed by diabetic patients presented anti-oxidative effects such as a considerable reduction in their TBARS (thiobarbituric acid reactive substances) and serum lipid peroxides by 28% and 56%, respectively, and the oxidative status of their monocytes/ macrophage levels. These effects belong specifically to presence of anthocyanins as highlighted in a vitro study by (Gil, Tomás-Barberán et al. 2000). Anthocyanin can work to inhibit various inflammatory mediators, such as IL-1β, TNF-α and the transcription factor NF-$_k$B leading to decrease of lipopolysaccharide LPS-induced reactive oxygen species ROS-mediated neuroinflammation (Khan, Ali et al. 2016) in addition to reduce the level of the oxidative stress kinase phospho-c-Jun N-terminal Kinase 1 (p-JNK).

Insulin Secretion

The task of insulin is to maintain a normal level of blood glucose by the stimulation of glucose uptake and its metabolism or suppression of glucose output from the liver (Jayaprakasam, Vareed et al. 2005) , in this context, inadequate generate of insulin or lack of insulin uptake at the tissues led to elevated glucose levels in the blood which is correlated with diabetes. It is quite known that dietary antioxidants such as anthocyanins, preserve cells of pancreatic beta from glucose-induced oxidative stress (Jayaprakasam, Vareed et al. 2005). (Jayaprakasam, Vareed et al. 2005) investigated the effect of anthocyanins and anthocyanidins in vitro, particularly the cyanidin, delphinidin and pelargonidn glycosides on glucose-induced insulin released from pancreatic beta-cells, where these phenolic compounds are considered the major bioactive components of Cornus fruits (European and Asiatic Cornelian cherry). The results proposed that both anthocyanidins and anthocyanins are insulin secretagogues (enhance secretion). The most effective was delphinidin-3- glucoside which sufficiently promote the insulin secretion at concentration of glucose (4 and 10 mmol/L) in comparing with the untreated cells. However, Cyanidin-3-glucoside was more effect at lower concentrations, while pelargonidin-3- galactoside was the lowest insulin secretor. These results demonstrate that hydroxyl group's number in ring B of anthocyanins (**Figure.1**) play a significant role in their ability to secrete insulin. According to recent studies it has also been confirmed the anti-diabetic activity of anthocyanin including decreasing the increase of blood

glucose level where (Jeon, Kang et al. 2018) reported that insulin level in mice serum treated with aronia berry extract (rich in anthocyanin) was significantly higher than non-treated.

(Zhang, Jayaprakasam et al. 2004) also reported that several compounds present in whole grapes or grape skin are able to enhance insulin secretion as well as selectively prohibiting cyclooxygenase-2 enzyme. Previous studies showed that the inducible cyclooxygenase-2 enzyme is related to inflammatory conditions (Zhang, Jayaprakasam et al. 2004). Thus, grape skins and wine waste product can play a big role as food additives with beneficial health effects.

Vasoprotective Effects

Diverse vasodilator agonists decrease endothelium dependent vasorelaxation in various pathological conditions including diabetes (Andriambeloson, Kleschyov et al. 1997). One of the essential mechanisms accounting for the dysfunction of the endothelium is a reduced release of nitric oxide (NO). Red wines extracts, other grape produce and various plants containing anthocyanins can promote endothelium-dependent vasorelaxation, via release of NO or enhanced biological activity of it, for example, red wine polyphenolic compounds have been demonstrated to promote the synthesis of NO resulting in stimulated endothelium-dependent relaxation in rat aorta (Andriambeloson, Kleschyov et al. 1997). Vasorelaxtion by grape products has been demonstrated to be mediated by the NO-cGMP (guanosine 3',5'-cyclic monophosphate) pathway (Fitzpatrick, Hirschfield et al. 1993). It has also been demonstrated that blackcurrant concentrate tend to promote endothelium-dependent vasorelaxation on smooth muscle of rat thoracic aorta (Nakamura, Matsumoto et al. 2002). This study showed that blackcurrant concentrate (10-20 ug/mL contain 10.83% anthocyanins) dose-dependently alleviated the norepinephrine- preconctracted aorta, but the scavenger oxyhemoglobin of NO abolished the vasorelaxation. The vasorelaxation promoted by blackcurrant concentrate could be happened by the H_1-receptors of histamine on the endothelium; however, the identification of anthocyanins into extracts which having this vasorelaxation activity was not clarified in these studies. More research is needed to validate these results by implement experiments about vasoprotective effects in vivo. Generally, studies propose that anthocyanins, as a functional food component, can assist in the prevention of diabetes and obesity.

NO produced by endothelial-type nitric oxide synthase (eNOS) is considered as a principal element in the vasoprotective function of the endothelium under physiological conditions (Li and Forstermann 2009, Förstermann and Sessa 2011) , in this contest, anthocyanin my contribute to beneficial effects to vasoprotective as (Xia, Pautz et al. 2014) reprted that treatment of human coronary artery smooth muscle cells (HCASMC) with four renowned artichoke compounds (cynarin > cyanidin > luteolin ≈ cynaroside) led to a downregulation inducible nitric oxide synthase iNOS mRNA and eNOS-upregulating.

Conclusion

According to the results of scientific research in recent decades, it has been concluded that anthocyanin is an important and influential material on human health, in addition to some applications in the field of food industry. However, the mechanism for the precise impact of anthocyanin on human body has not been identified so far and the research is still continuing to discover more anthocyanin compounds found in different varieties of plants.

In Future Approaches, Scientists seek greater benefits for anthocyanins similar to the effects of enzyme SOD (Superoxide dismutase) in relieving pain of inflammation and reperfusion. Not far from the applications of biotechnology and its research in genetics where scientists try to produce plants containing higher percentages of anthocyanins and antioxidants (Devasagayam, Tilak et al. 2004). (Suntres 2011) reports to the possibility of producing antioxidants with protective and therapeutic effects through the selective effect of specific sites within living organisms.

It is true that antioxidants are advantageous and play a useful role in human homeostasis, therefore it is necessary to achieve a productive work in the academic community and should search deeper into the kinetics and *in vivo* mechanisms of antioxidants to detect the optimal concentrations and desired functions in order to get an advanced steps against cardiovascular, neurodegenerative and cancer diseases.

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Appendices

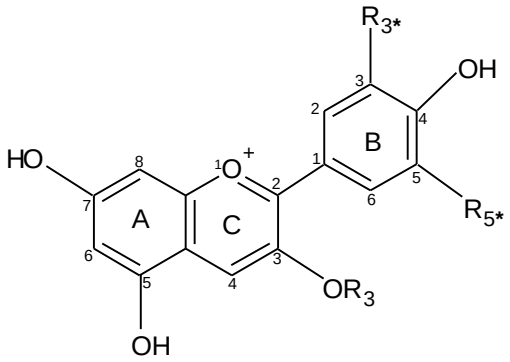
Table 2 some most important anthocyanin compounds in various diferente kinds of food

Anthocyanin	Food cultivar	References
Myricetin, quercetin and methoxy quercetin	Cranberry (Vaccinium macrocarpon L.)	(Oszmiański, Kolniak-Ostek et al. 2015)
Malvidin 3-galactoside, delphinidin 3-galactoside	highbush	(Li, Li et al. 2017)
Delphinidin 3-glucoside, cyanidin 3- diglucoside, 3,5-diglucoside, pelargonidin 3-glucoside and 3,5 diglucoside	beans of *Canadian Wonder* cultivar	(Stanton and Francis 1966)
3-*O*-glucoside malvidin 3-*O*-glucoside, and petunidin 3-*O*-glucoside.	seed coat beans	(Takeoka, Dao et al. 1997)
Pelargonidin 3-glucoside	black-seeded soybean varieties	(Yoshikura and Hamaguchi 1969)
Cyanidin 3-*O*glucoside and cyanidin3-*O*-rutinoside	olive fruits	(Romani, Mulinacci et al. 1999)
Cyanidin-3-*O*-glucoside, Cyanidin-3-*O*-rutinoside, Delphinidin-3-*O*-glucoside, Delphinidin-3-*O*-rutinoside, Petunidin-3-*O*-glucoside, Malvidin-3-*O*-rutinoside.	bilberry fruit	(Chandra, Rana et al. 2001)
Cyanidin-3-galactoside, Cyanidin-3-glucoside, Cyanidin-3-arabinoside, Cyanidin-3-xyloside, Malvidin-3-arabinoside	blackberry	(Sellappan, Akoh et al. 2002)
cyanidin 3- glucoside	Blackcurrants	(Iversen 1999)
Cyanidin-3-glucoside	blueberry	(Ehlenfeldt and Prior 2001)

3-*O*-galactosides and 3-*O*arabinosides of cyanidin and peonidin	Cranberries	(Mazza 2018)
Cyanidin 3-glucoside; cy 3-acetylglucoside; cy 3-p-coumaryl-glucoside; peonidin 3-glucoside; pn 3-acetylglucoside; pn 3-p-coumarylglucoside; pn 3-caffeylglucoside; delphinidin 3-glucoside; dp 3-acetylglucoside; dp 3-p-coumarylglycoside; petunidin 3-glucoside; pt 3-pcoumarylglucoside; malvidin 3-glucoside; mv 3-acetylglucoside; mv 3-p-coumaryglucoside; mv 3-caffeylglucoside	*Vitis Vinera* Berries	(Goupy, Varoquaux et al. 1990)
cyanidin 3-rutinoside	Grape (*Flame* variety)	(Cantos, Espin et al. 2002)
Cyanidin 3-glucoside and cyanidin 3-rutinoside	Nectarines and peaches	(Mazza 2018)
Cyanidin 3-glucoside and cyanidin 3-rutinoside	Plums	(Hong and Wrolstad 1990)
cyanidin 3-galactoside and cyanidin 3-arabinoside	Pears	(Timberlake and Bridle 1971)
pelargonidin 3-diglucoside and delphinidin 3-glucoside	Passion Fruits	(Harborne 1967)
pelargonidin 3-glucosides, pelargonidin 3,5- diglucosides of, delphidin and cyanidin 3-glucoside	pomegranate	(Du, Wang et al. 1975, Gil, Tomás-Barberán et al. 2000)
cyanidin 3-glucoside, peonidin 3-glucoside and cyanidin 3-laminariobioside	Red onion	(Herrmann 1976, Gennaro, Leonardi et al. 2002)

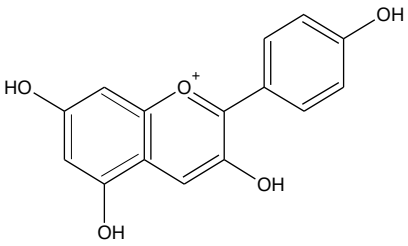
Table 3 The content of total Anthocyanin in Cranberry Fruit Cultivars Stored for 3 Months at Different Storage Temperatures, Milligrams of cyanidin 3-O-galactoside per kilogram of fresh weight (Wang and Stretch 2001).

	Storage Temperature, °C			
Cultivar	Initial	0	5	15
Ben Lear	250±13	460±25	546±29	766±35
Cropper	198±13	348±16	492±30	604±21
Crowley	656±20	654±34	808±37	1092±29
Early Black	634±15	724±52	886±41	1172±32
Franklin	541±36	604±18	696±32	936±14
Howes	235±11	330±14	494±16	710±22
Pilgrim	207±11	308±15	386±10	600±23
Stevens	228±13	316±12	474±25	656±18
Wilcox	243±17	548±27	646±28	724±17

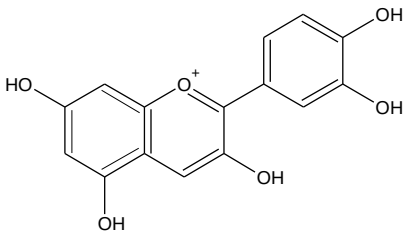


Anthocyanin	R_3	R_{3*}	R_{5*}
Cyanidin	H	OH	H
Pelargonidin	H	H	H
Delphinidin	H	OH	OH
Peonidin	H	OCH_3	H
Petunidin	H	OCH_3	OH
Malvidin	H	OCH_3	OCH_3
Pelargonidin 3-glucoside	Glc	H	H
Cyanidin 3-glucoside	Glc	OH	H
Delphinidin 3-glucoside	Glc	OH	OH
Peonidin 3-glucoside	Glc	OCH_3	H
Petunidin 3-glucoside	Glc	OCH_3	OH
Malvidin 3-glucoside	Glc	OCH_3	OCH_3

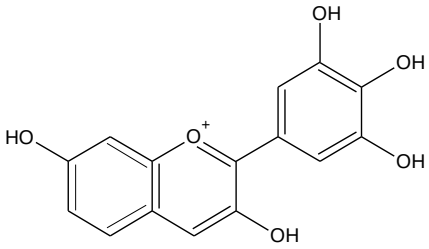
Figure 2 Major structures of anthocyanins (Giusti, Rodríguez-Saona et al. 1999)



Pelargonidin



Cyanidin



Delphinidin

Figure.2. Chemical structures of anthocyanins.

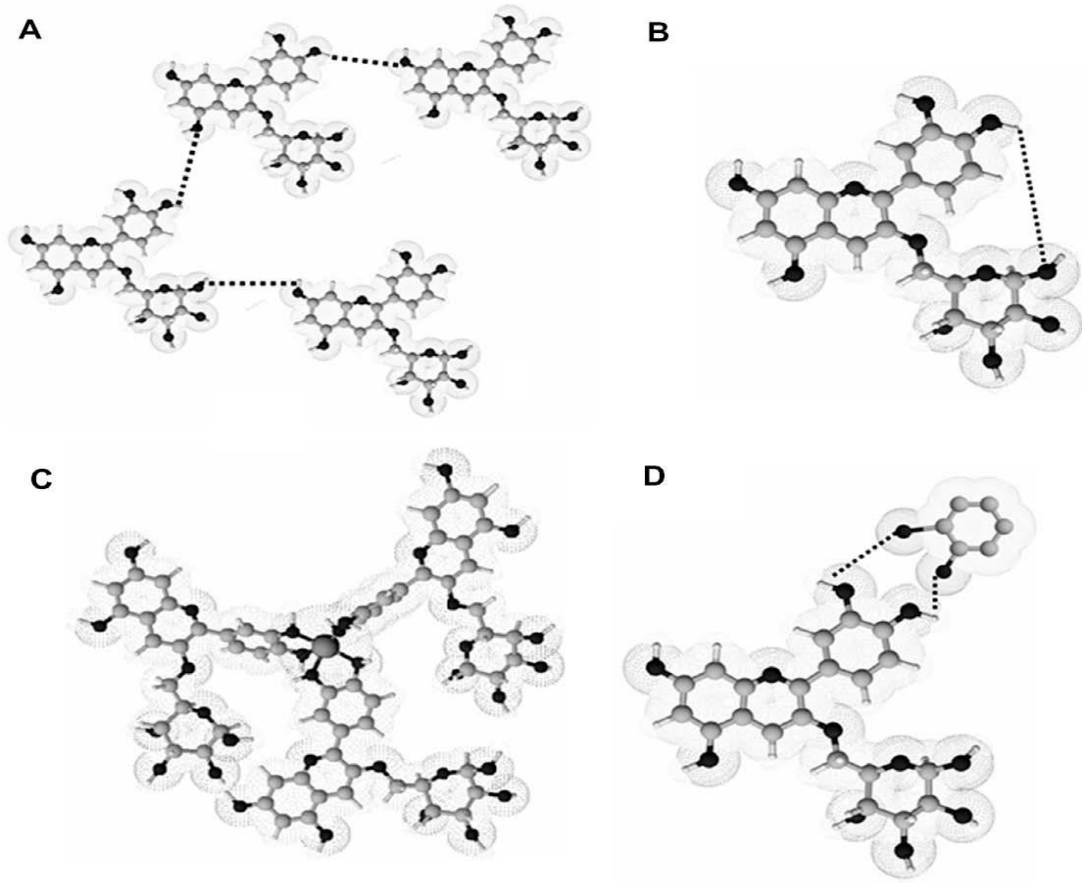
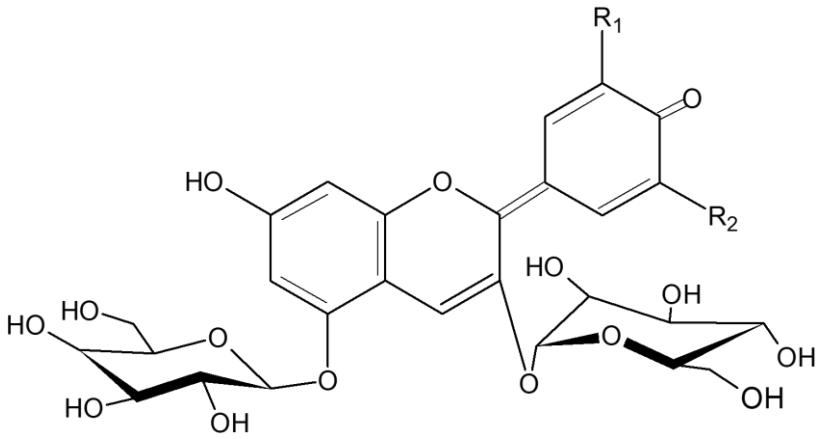


Figure.3. Interaction of anthocyanins: Self-association [A], intramolecular copigmentation [B], metal complexation [C], intermolecular copigmentation [D] (Castaneda-Ovando, de Lourdes Pacheco-Hernández et al. 2009).



R_1	R_2	Anthocyanin	Aglycon
H	H	Pelargonin	Pelargonidin
OH	H	Cyanin	Cyanidin
OCH_3	H	Peonin	Peonidin
OH	OH	Delphin	Delphinidin
OCH_3	H	Petunin	Petunidin
OCH_3	OCH_3	Malvin	Malvidin

F ig.4. Structure of common anthocyanins

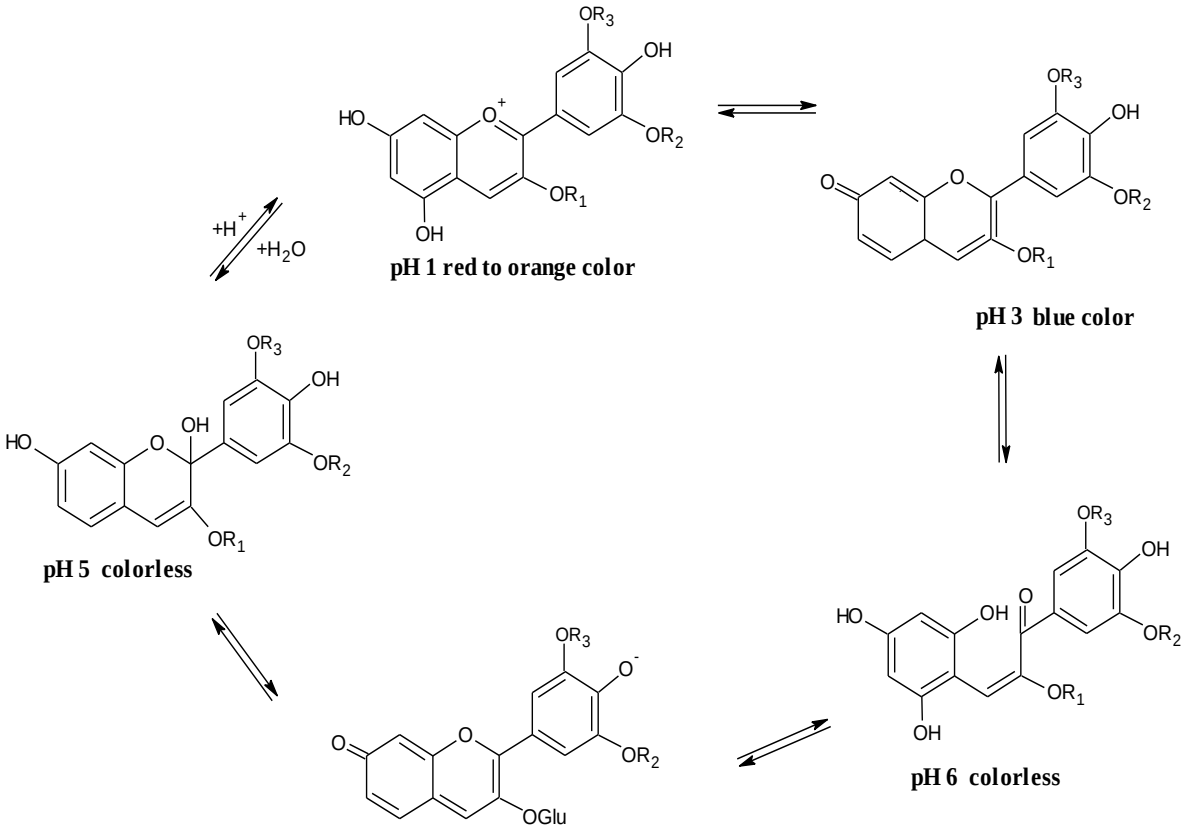


Figure.5. Anthocyanins chemical structures and colours depending on pH , Where (R_1 = H or glycoside;, R_2 and R_3 = H or Methyl group) (Castaneda-Ovando, de Lourdes Pacheco-Hernández et al. 2009).

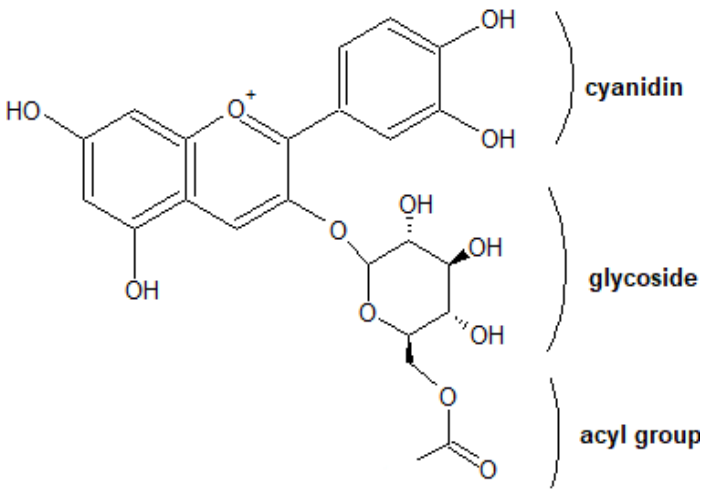
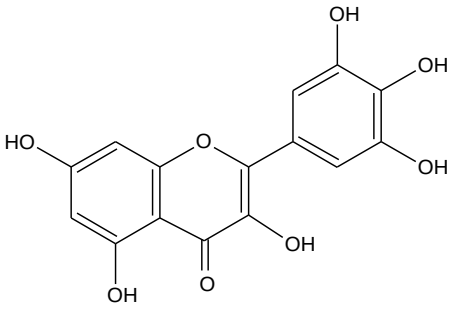
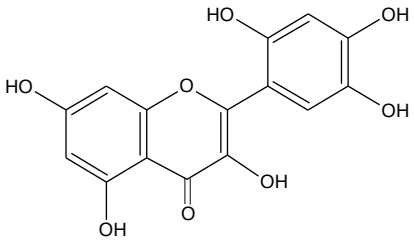


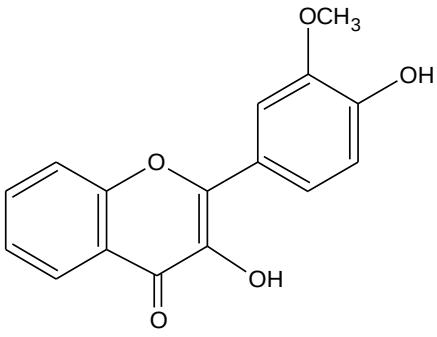
Figure.6.the skeletal structure of an acylated anthocyanin



Myricetin



Morin



Isorhamnetin

Figure.7. Chemical structures of myricetin, morin and isorhamnetin

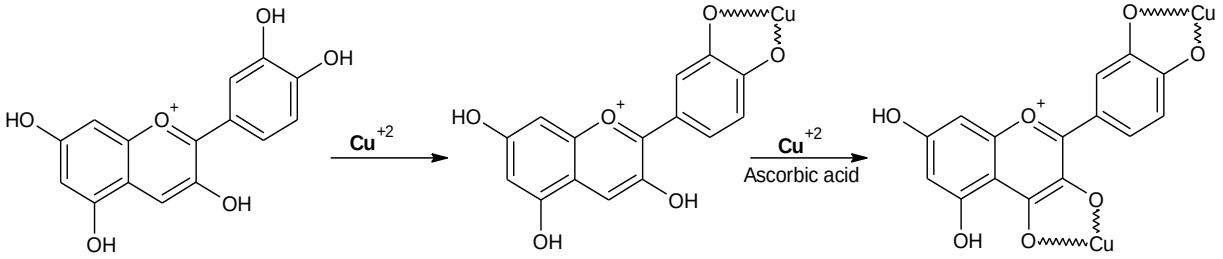


Figure.8. A possible mechanism of increased metal chelaring by cyanidin in presence of copper and ascorbic acid.

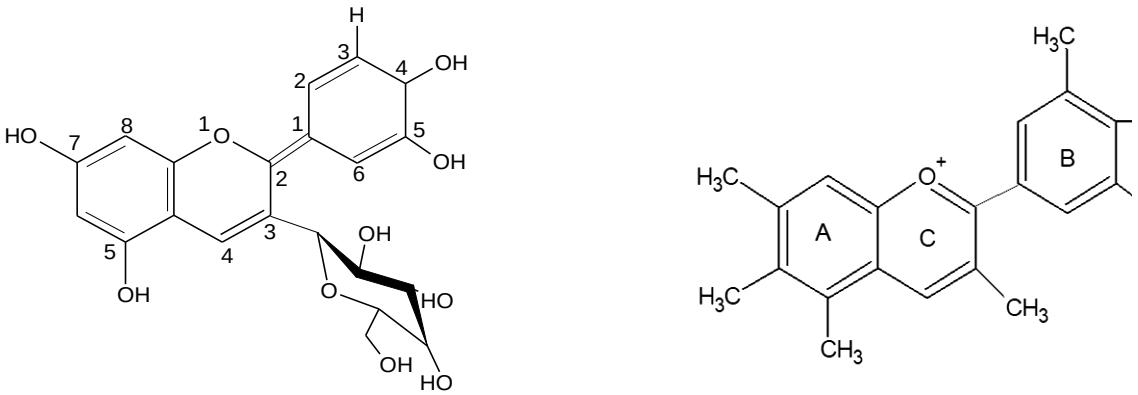


Figure.9. the flavylium cation and anthocyanin

CAPÍTULO II: Adsorption/desorption characteristics and separation of anthocyanin compounds from grape wine pele pomace *Alicante Bouschet* using microporous resins

Adsorption/desorption characteristics and separation of anthocyanin compounds from grape wine peel pomace *Alicante Bouschet* using microporous resins

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Abstract

Grapes are one of the most commonly produced fruit crops in the world. Consequently, the grape industry produces large amount of grape pomace which is a rich source of health promoting polyphenols such as proanthocyanidins, flavones and flavan-3-ols, in this context, there are many types of adsorbents available, but macroporous resins are reported to be the most efficient for polyphenol recovery because of its characteristics such as low cost, high recuperative capacity and long term-validity. Therefore, in this study, the adsorption/desorption characteristics of anthocyanins of grape wine peel pomace *Alicante Bouschet* (GWPP) on six types of macroporous resins (XAD 2, XAD 4, XAD 7HP, XAD 8, XAD 11 and XAD 16 were investigated. On basis of static adsorption test XAD-7HP and DAX 8 showed higher adsorption/desorption capacities. The adsorption mechanism indicated that the process was better explained by pseudo-first-order kinetics and the Langmuir isotherm model. Adsorption isotherm tests showed that the adsorption process was exothermic on XAD 7HP and DAX 8 when performed at 30° C compared to 40 °C and 50 °C. The results of antioxidant tests indicate that purified anthocyanin extract showed high antioxidant capacity in the both methods of FRAP and DPPH 12.75 and 12.06 mg TE.g^{-1} respectively. Regarding to high-performance liquid chromatography analysis, the initial and purified extracts were rich in acylated anthocyanins (67.06%) and (71.33%) respectively. Overall, macroporous resins can be an effective tool to purificate anthocyanin instead of using organic solvents, in addition to supply pharmaceutical and food industry with safe and cheap process.

Keyword: antioxidant, cinetic test, DPPH, purification, polyphenol.

1. Introduction:

The grape of *Alicante Bouschet* variety is considered one type of red-flesh grape riched into anthocyanin that is produced in Brazil (Castillo-Munoz, Fernandez-Gonzalez et al. 2009), Total phenolic content range of (951.8 to 4038.9 mg gallic acid equivalent GAE/100 g of grape skin) can be found in *Alicante Bouschet* and make this latter rich in photochemical and antioxidant content that play a big role within organism (Ruíz-García, Beres et al. 2019).

Grapes are one of the most commonly produced fruit crops in the world, with approximately 75 million tons produced each year. It is also one of the most abundant fruits: while almost 80% of grapes are used to make wine, one third is consumed as fresh fruit and the rest is dried, consumed as grape juice or stored in the form of grape musts (whether concentrated or not) (Fontana, Antoniolli et al. 2013, Moncayo and Aurand 2016). Consequently, the grape industry produces large amounts of grape pomace that are a rich source of health-promoting flavonoids such as proanthocyanidins, flavones and flavan-3-ols (Cheynier and Rigaud 1986); such as flavonoids (quercetin, kaemferol, myricetin), catechins or phenols (carnosol, rosmanol, rosamaridiphenol) and phenolic acids (carnosic acid, rosmarinic acid) (Brazinha, Cadima et al. 2014, Caldas, Mazza et al. 2018).

At the end of the manofacturing process, much quantity of remains are being discharged containing high amount of phenolic compounds including anthocyanins, catechins, flavonol glycosides, phenolic acids and stilbenes (Kammerer, Kammerer et al. 2014). This is considered from view of the environmental management authorities as a serious threat because they are extremely contain acidify and organic materials thus potentially causing a phytotoxic effect if reached to crops or wetlands(Lavelli, Harsha et al. 2017). Therefore, proceeding with a process to convert and employ this by-product to another useful product would be an effective solution to this environmental dilemma.

Anthocyanins are also used as nutrients in many kinds of food in the form of powder extracted from fruits, these compounds can be extracted and used in food industries (Bridle and Timberlake 1997, Mazza 2018). Many studies were implemented about phenolic compounds and anthocyanins due to their important role for health and food quality. These phytochemicals are associated with counteracting the risk of cardiovascular diseases, cancer and cataract as well as a great number of other degenerative diseases (Singleton 1981, Hollman 2001, Su, Wang et al. 2018),

At present, high purity of phenolics is greatly needed in the field of pharmacy and functional food. Conventional extraction methods such as maceration and Soxhlet have shown low efficiency and potential environmental pollution due to large volumes of organic solvents and long extraction time required (Dai and Mumper 2010). Therefore, it is necessary to study simple and efficient environmentally friendly methods to extract polyphenols. With the trends of green chemistry, a new class of promising solvents to replace volatile organic solvents in samples preparation has emerged (Wei, Qi et al. 2015), in this context macroporous resin MARs are porous cross-linked polymer beads that have been developed as useful adsorbents. They are considered more applicable than common adsorbents due to their favorable physical and chemical stabilities, large surface areas, easy regeneration, long service life (Chen, Zhang et al. 2010) , fast adsorption rate, strong adsorption capacity, and easy elution , physicochemical stability, high adsorption selectivity and easy recycling (Wan, Sheng et al. 2014).

Several studies had looked into adsorption of gallic acid (GA) and licorice flavonoids on XDA-1 resin (Fu, Liu et al. 2005), but the main focus of their work was to remove GA in order to obtain a deglycyrrhizinated licorice product. Although there are studies on the extraction of phenolic compounds of grape pomace, none study separation of anthocyanin compounds from grape wine peel pomace *Alicante Bouschet* using macroporous resins.

2. Methods and materials

2.1. Chemicals

Sodium acetate, hydrochloric acid, potassium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and organic solvents were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Materials:

The raw material used was the grape peel pomace *Alicante Bouschet* from the production of red wine, supplied by the *Rio Sol winery* of the *ViniBrasil group* (*Lagoa Grande, PE*). The pomace was subjected to convective drying at 60 °C for 24 hours to get a dried solid pomace. The pomace was also milled in a disc mill (DM) LM3600 (Perten instruments, Huddinge, Sweden) and sieved in a ROTAP RX-29-10 (W.S. Tyler, St, Albans, USA) to get particles with size between 75 and 425µm and save it in freezer at -20 °C.

Amberlite resins (XAD 16, XAD 7HP, XAD 2, XAD 4, DAX 8 and XAD 1180N) were purchased from Sigma-Aldrich (St. Louis, Missouri, EUA). The chemical and physical properties are summarized in Table 1.

Table 1. Chemical and physical properties of resins.

Amberlite resins	Chemical matrix	Polarity	Surface área ($m^2.g^{-1}$)	Pore envelope (Ã)
XAD 16	Styrene-divinylbenzene	nonpolar	800	200
XAD 7HP	Acrylic ester	polar	500	450
XAD 2	Styrene-divinylbenzene	nonpolar	300	90
XAD 4	Styrene-divinylbenzene	nonpolar	750	100
XAD 1180N	Styrene-divinylbenzene	nonpolar	500	400
DAX 8	Acrylic ester	moderalety polar	160	225

2.3. METHODS

2.3.1. Preparation of Organic Grape Pomace GP Extracts

Dried GP (1 g) was weighted and mixed with 12.5 mL of 50% water and 50% ethanol, the mixture was put in a shaker at room temperature for one hour. The mixture was decanted and filtered through *Whatmann No.5* filter paper, similarly the residue was rextracted by same volume of 12.5 mL solution and the filtered extracts were stored in freezer at -20 °C.

2.3.2. Pre-Treatment of Resins

The pre-treatment of resins were performed according to the procedures written by (Buran, Sandhu et al. 2014). Five grams of resins were firstly soaked in water, and treated in ethanol (140 mL, 95%). Resins were then washed with water until the eluent is clear, and eluted with 140 mL of 4% HCl. After that, resins were washed with distilled water until neutral pH and then washed with 140 mL of 5% NaOH, followed by distilled water until a pH of 7.0. Lastly, all the resins were dried at 60 ˚C in an oven for 24 h to reach a constant weight for moisture determination.

2.3.3. Static Adsorption and Desorption Tests

Pre-treated resins (0.5 g) and 12.5 mL of grape wine pele pomace (GWPP) extracts were added to a 125 mL Erlenmeyer flask, the flasks were taken to a shaker at 100 rpm for 24 h at 30 °C. For static desorption test, the resins were filtered, washed with distilled water and added to 50 mL of ethanol (95%). The flasks were kept in a shaker at 100 rpm for 24 h at 50 °C. Adsorption and desorption ratios and capacities were determined according to the following equations:

Adsorption ratio: $A\ (\%) = \frac{(C_o - C_e)}{C_o}$ Equation (1)

Adsorption capacity: $q_e = (C_o - Ce) \times \frac{(V_i)}{m}$ Equation (2)

Where: A is the adsorption ratio (%). qe is the adsorption capacity (mg/g dry resin) at equilibrium; C_o is the initial concentration of total anthocyanin in the extracts (mg/L); C_e is the equilibrium concentration of total anthocyanin in the extracts (mg/L); m is the initial weight of resin (g); V0 is the volume of extract used (mL).

Desorption ratio: $D\% = C_d \frac{v_d}{(C_d - C_e)V_0} \times 100$ Equation (3)

Desorption capacity: $q_d = C_d \times \frac{V_d}{m}$ Equation (4)

% Recovery: $R = \frac{C_d V_d}{C_o v_o} \times 100\%$ Equation (5)

Where: *D* is the desorption ratio (%); q_d is the desorption capacity (mg/ g dry resin); *R* is the recovery after complete desorption; C_d is the concentration of total anthocyanin in the desorption solution (mg/L); V_d is the volume of the desorption solution (mL); *Co*, *Ce*, *m* and V_0 are the same as those in Equations 1 and 2.

2.3.4. Adsorption Kinetics Test

According to the best results of adsorption ratio and adsorption capacity obtained at the best two *Amberlite* resins, DAX 8 and XAD 7HP were selected for adsorption kinetics and adsorption isotherms. Pre-treated resins (1 g) and 25 mL of GP extracts were added to a 125 mL Erlenmeyer flask. Flasks were taken to a shaker (100 rpm) at 30 °C for 48 h. An aliquot of 200 µL were taken out every 30 min for the first 3 hours, every 60 min from 3 to 6 hours, and then at 12 and 24 hours to measure total phenolics contents. Two kinetic models, pseudo first order and pseudo second order models were applied to fit the adsorption process.

Pseudo-first-order model: $qt = qe\{1 - e^{-Kf.t}]$ Equation (6)

Pseudo-second-order model: $\frac{t}{qt} = \frac{1}{ks.qe^2} + \frac{1}{qe}t$ Equation (7)

Where: Kf is the rate constant of the pseudo-first-order-model and Ks is the rate constant of the pseudo-second-order-model, qt (µg.g-1) is the amount of total phenolics adsorbed at time t and qe is the adsorption capacity at equilibrium.

2.3.5. Adsorption Isotherm Tests

According to static adsorption/ desorption and kinetics tests the best two types resin XAD 7HP and DAX 8 were selected to be studied at adsorption isothermal test, 0.5 g of pre-treated resin was added to 12.5 mL of pomace extracts of different concentrations of phenolic compounds (75, 100, 125, 150 and 174.449 μg.mL^{-1}) to a 125 mL Erlenmeyer flask. The adsorption was taken place at three temperatures (30, 40 and 50 ˚C) in a shaker at 100 rpm. Total anthocyanin in the solutions was quantified after 24 hours of adsorption. The equilibrium adsorption isotherms for anthocyanin were calculated using Langmuir, Freundlich and Henry equations:

Langmuir equation: $qe = \frac{qm.ql.ce}{1+kl.ce}$ Equation (8)

Freundlich equation: $q_e = K_F C_e^{1/n}$ Equation (9)

Henry equation: $qe = K.Ce$ Equation (10)

Where: *qm* is the maximum adsorption capacity (μg.g-1); K_l is constant of Langmuir model; K_F is the Freundlich constant that indicates the adsorption capacity; 1/n is an empirical constant related to the adsorption affinity of the adsorbent for the absorbate; K is the constant of Henry model.

2.3.6. Total anthocyanin and total monomeric anthocyanin determination:

Total anthocyanins and total monomeric anthocyanins were determined following the method by (Giusti and Wrolstad 2001). Absorbance of test portion diluted with potassium chloride buffer (pH 1.0**)** and sodium acetate buffer (pH 4.5) at both 520 and 700 nm were determined. The diluted test portions are read versus a blank cell filled with distilled water. The maximum test portion added should be (1 part test portion, 4 parts buffer) so as not to exceed the buffer capacity of the reagents. Absorbance was measured within 20–50 min of preparation.
Note: The reason for measuring the absorbance at 700 nm is to correct for haze. However, if the diluted test portion is excessively turbid, clarify by centrifuging or filtering before measurement. Use a filter (e.g., MilliporeTM membrane filter, ≤ 1.2 μm pore size, Millipore Corp., Bedford, MA) that will not absorb the anthocyanins.

The amount of total anthocyanins was expressed as cianidina 3 glicosídio using the following equation:

$\mathrm{Abs} = (\mathrm{Abs}_{510} - \mathrm{Abs}_{700})_{\mathrm{pH\ 1,0}}$

$\mathrm{C(mg/L)} = (\mathrm{Abs} \,.\, \mathrm{Mw} \,.\, \mathrm{Df}.\, 1000) \,/\varepsilon$

The amount of monomeric anthocyanins was expressed as cianidina 3 glicosídio using the following equation:

$\mathrm{Abs} = (\mathrm{Abs}_{510} - \mathrm{Abs}_{700})_{\mathrm{pH\ 1,0}} - (\mathrm{Abs}_{510} - \mathrm{Abs}_{700})_{\mathrm{pH\ 4,5}}$

$\mathrm{C\ (mg/L)} = (\mathrm{Abs\ x\ Mw\ X\ Df\ x\ 1000}) \,/\, \varepsilon$

Mw (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); Df = dilution factor established in D; l = pathlength in cm; (= 26 900 molar extinction coefficient, in L × mol^{-1} × cm^{-1}, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

2.3.7. High Efficiency Liquid Chromatography (HPLC)

The samples of (GWPP) extract was injected into a high efficiency liquid chromatograph (HPLC) Alliance 2695 (Waters, Milford, USA) and a W2996 array photodiode array detector (PAD). The column used was Thermo® (BDS Hypersil C18, 4.6 × 100 mm, 2.4 μm). Gradient elution method with acetonitrile and 5 % formic acid was used (Gouvêa, Araujo et al. 2012). The injection volume of 5 μL, a run time of 30 min. The readings were performed between 270 and 535 nm.

The quantification of the main anthocyanins was estimated based on calibration curve using external standardization of malvidin-3-*O*-glucoside (Gouvêa, Araujo et al. 2012)

2.3.8. FRAP Tests

The FRAP method measures the iron reduction of 2,4,6 tripyridyl-S-triazine (TPTZ). It was performed according to Thaipong et al. (2006) with modifications; 90 μL of grape wine peel pomace extract was diluted in water (270 μL), to this mixture 2.7 mL of the FRAP reagent was added. This mixture was vigorously stirred and allowed to stand in a heating bath at 37 ° C for 30 min in the absence of light. Afterwards, the absorbance was read at 595 nm in a spectrophotometer. The results were expressed in Trolox equivalent using Trolox antioxidant standards

2.3.9. DPPH Tests

The ability to sequester the DPPH radical will be determined according to Rufino et al. (2010), with modifications; DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical which accepts an electron or a hydrogen radical to become a stable diamagnetic molecule and thus is reduced in the presence of an antioxidant. In the presence of an antioxidant, the purple staining of DPPH decays and the change in absorbance can be read spectrophotometrically. (150 μL) of each grape wine pomace extract was mixed to 2.85 mL of a 0.06 mM DPPH solution. The mixture was stirred vigorously and allowed to stand for 1 h in the dark, after which time the spectrophotometer was read at 517 nm and results were expressed in Trolox (TE) equivalent using Trolox antioxidant standards.

2.3.10. Statistical Analysis

All tests were performed in duplicate. The experimental data were expressed as mean ± standard deviation and were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test at 5% of significance.

The experimental models of kinetics and the adsorption / desorption isotherm were adjusted using the non-linear regression method using the Statistica 7.0 program. The adequacy of the model between the experimental and predicted data was verified based on the coefficient of determination (R^2).

3. Results and discussion

3.1. Static adsorption and desorption

Synthetic resins allow adsorption of polyphenols from aqueous solution via hydrophobic binding and aromatic stacking. They desorb phytochemicals in organic solvents, such as methanol or ethanol (Buran, Sandhu et al. 2014).

Different condition can effect on adsorption and desorption capacity including type of resin, temperature and composition of sample solution (Wang, Wang et al. 2017)

Adsorption and desorption of total anthocyanin and total monomeric anthocyanin on different kinds of resins are presented in Figure 1 and Figure 2. Amberlite resins XAD 7HP, DAX 8 and XAD 16 whose surface area along with pore size were higher had the highest adsorption capacity and adsorption ratio, similarly, previous research suggests that the surface area along with pore size were the decisive factors reflecting the adsorption capacity (Chandrasekhar, Madhusudhan et al. 2012, Chen, Zhang et al. 2016).

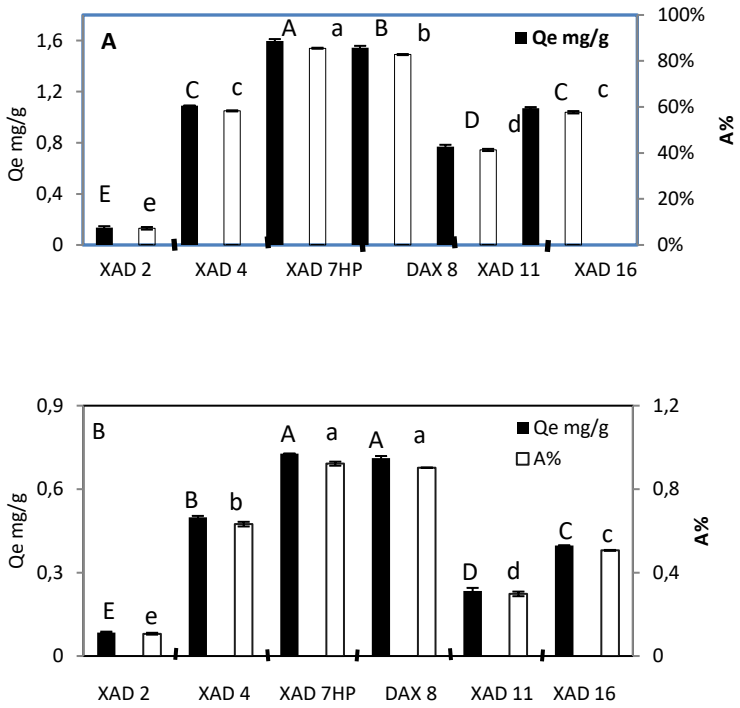

Figure1.Static adsorption capacity Qa (mg.g^{-1}) and adsorption ratio A (%) of total anthocyanin (A) and total monomeric anthocyanin (B) from grape wine pomace using different macroporous resins. Results are mean of two determinations. Different upper-case letters indicate significant differences of black bars. Different lower-case letters indicate significant differences of white bars ($p \leq 0.05$).

Comparing the six resins, XAD 7HP had the highest adsorption capacity of TA (1.596.mg. g^{-1}) and (726.998 mg. g^{-1}) of TMA which could be attributed to their similarity to the polarity of anthocyanins that primarily affected by the three sections of an anthocyanin: (1) the anthocyanidin; (2) number and type of attached glycosides; and (3) any attached acyl groups, in addition to the high surface area of adsorbents. (Jampani, Naik et al. 2014, Yang, Yuan et al. 2015, Chen, Zhang et al. 2016).

XAD 2 presented the lowest adsorption capacity of TA (0.135 mg. g^{-1}) and (84.605 mg. g^{-1}) of TMA , the slightly low adsorption capacity of XAD 2 may be explained by its small pore size, small surface and non-polar property as shown in table 1.

Desorption percentages of resins are showed in figure 2 with lower desorption percentage (D = 31%) of TA and (31%) of TMA for XAD 2 resin in comparison with the other six resins while XAD 16 presented the highest desorption percentage of TA among these six resins, furthermore, there wasn´t a difference significant between these resins except XAD 2. for TMA.

These results are corroborated in the Figure 2C, which showed that the highest percentage of TA and TMA recovery (%R) is observed in XAD 7HP (81.7%, 57%) respectively and DAX 8 (78.9%, 60%) respectively.

Similar results was reported by (Silva, Pompeu et al. 2007) (Chen, Zhang et al. 2016) that XAD 7 showed the bests results regarding the adsorption processo of phenolics from *Inga edulis* leaves .Static tests indicated that XAD 7HP and DAX 8 were more efficient at adsorption and desorption than other resins. Therefore, they were chosen for further kinetic tests.

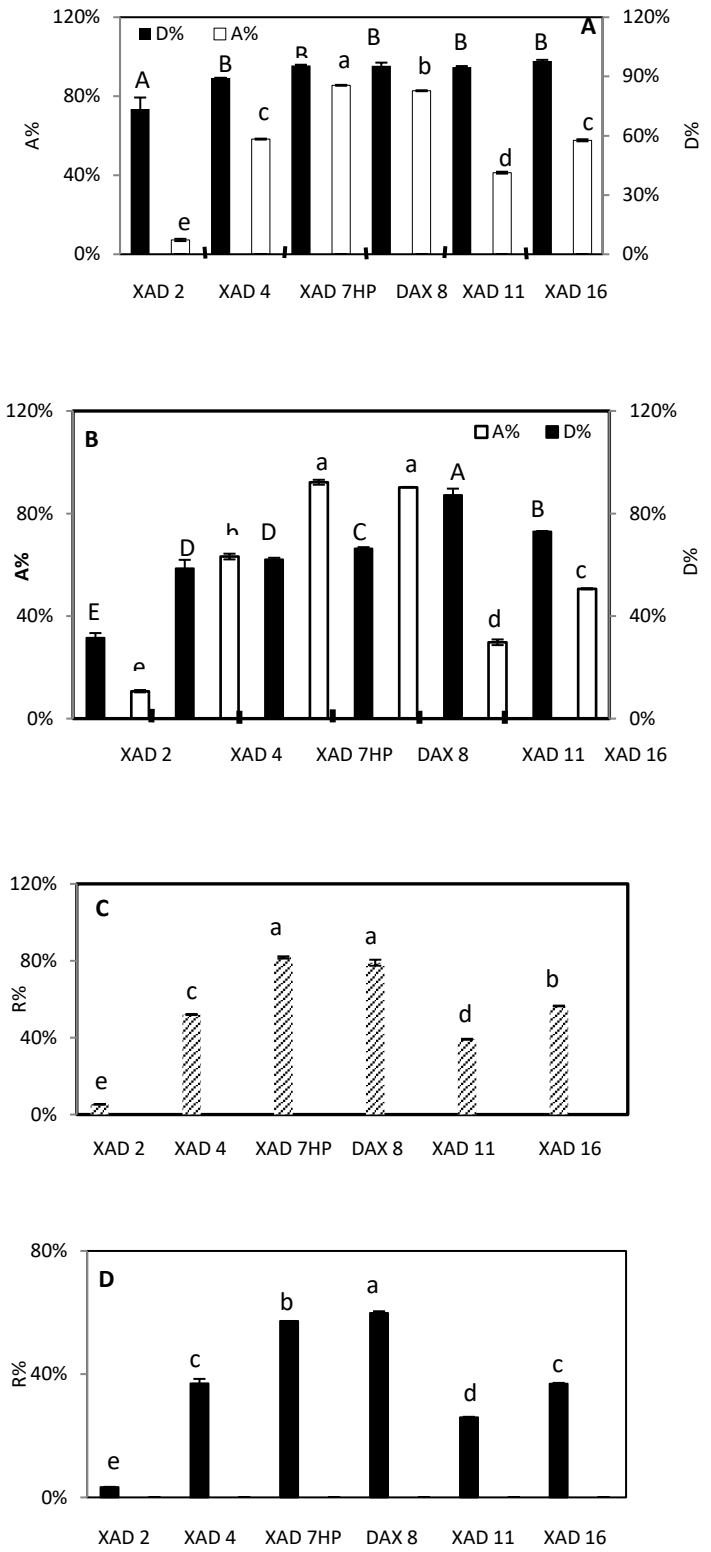

Figure 2. Static adsorption and desorption of TA (A), TMA (B) , recovery ratio (C) of TA and (D) recovery ratio of TMA from grape wine pomace using different macroporous resins. Different upper-case letters indicate significant differences of black bars. Different lower-case letters indicate significant differences of white bars ($p \leq 0.05$).

3.2. Adsorption kinetics test

Adsorption kinetics of total anthocyanin on DAX 8 and XAD 7HP are represented in Figure 3. The adsorption of total anthocyanin on DAX 8 at 30 °C, 40 °C and 50 °C reach equilibrium after 4 hours. The adsorption of total anthocyanin on XAD 7HP at 30 °C, 40 °C and 50 °C reached equilibrium approximately at the same time after 4 hour. Although DAX 8 needed similar time to reach the adsorption equilibrium, it presented lower adsorption capacity at equilibrium for the both kinetic models, pseudo first order and pseudo second order models, when compared with XAD 7HP as shown in Table 2. The ideal resin should provide higher adsorption capacity and shorter processing time.

The adsorption rate and adsorption capacity are strongly affected by temperature as shown in the Figure 3 the adsorption rate vary directly with temperature and the adsorption capacity vary inversely because the adsorption is an exothermic process.(Benefield, Judkins et al. 1982). As illustrated in Figure 3, the adsorption capacity decreases with the temperature increasing from 30 to 50 °C at the same initial concentration, which corroborates that the adsorption process is an exothermic process. Similar results were obtained for the recovery of phenolics using macroporous resin (Nathália da R. Rodrigues, Junior et al. 2018) (Zhu, Song et al. 2017, Wu, Han et al. 2018). For this reason, 30 °C was selected in the following experiments.

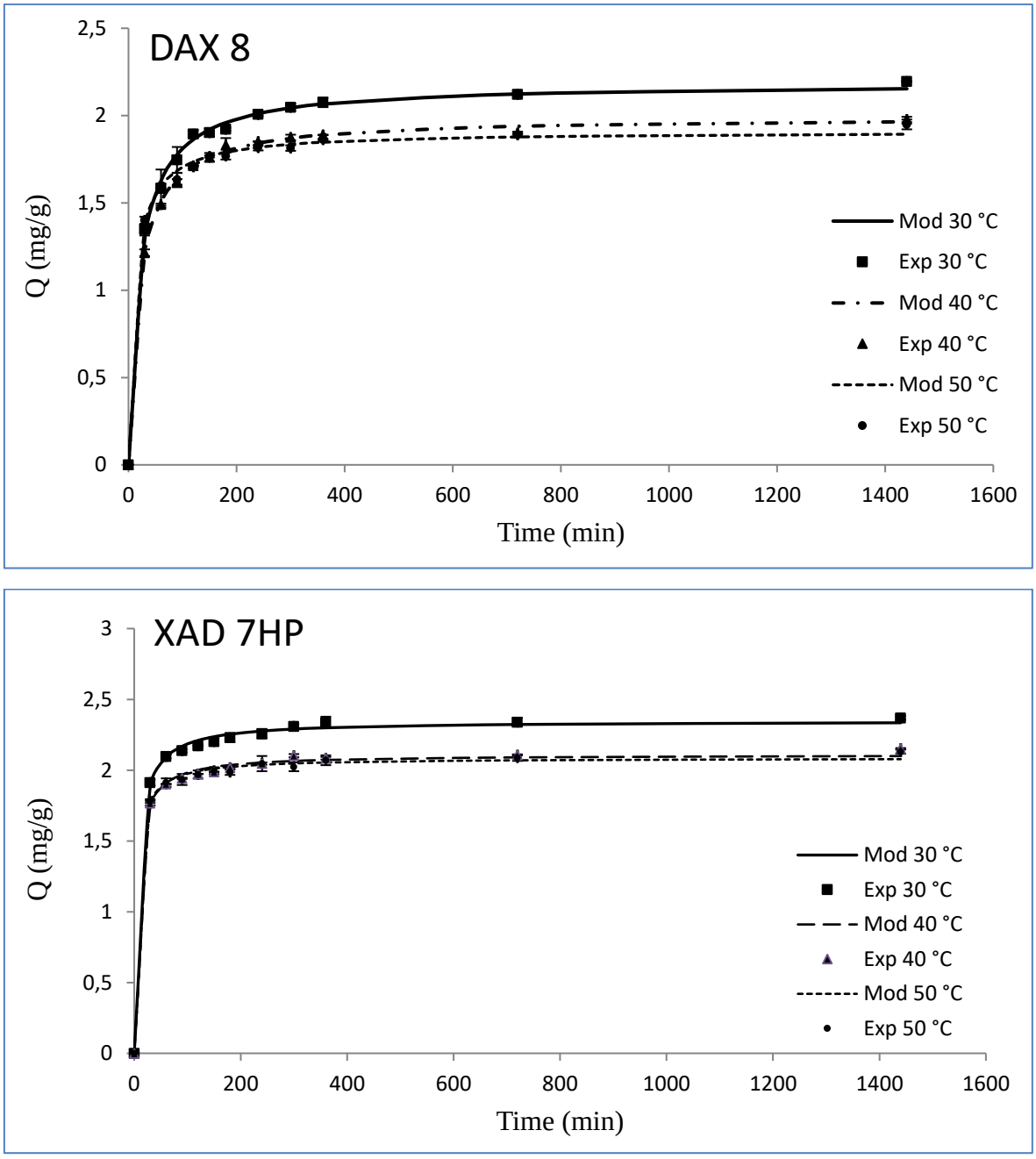

Figure 3. Adsorption kinetics of total anthocyanin from GWPP extracts (Q= adsorption capacity; Exp= experimental values; Mod= pseudo-second-order model values). Results are mean of two determinations

Both pseudo-first and pseudo-second-order models have been applied for adsorption kinetics studies. The pseudo-first order model is generally applicable over the initial stage of an adsorption process, while the pseudo-second-order model assumes that the rate-limiting step is chemisorption and predicts the behavior over the whole range of adsorption (Chang, Wang et al. 2012).

The adsorption linear regression and rate constants values derived from pseudo first-order and pseudo second-order kinetic equations are summarized in Table 2. For XAD 7HP the correlation coefficients (R^2) of pseudo first order model were 0.9934 0.994 and 0.995 at 30° C, 40° C and 50º C respectively and the (R^2) values of pseudo second-order model were 0.999, 0.9988 and 0.9989 at 30° C, 40° C and 50º C respectively, similarly the (R^2) values of pseudo second-order

model for DAX 8 were more than first order model. Thus, pseudo second-order model had a better capacity than pseudo first-order model to predict adsorption capacity of anthocyanins. Similar results have also been reported in previous studies using macroporous resins for the adsorption of anthocyanins from different resources (Chang, Wang et al. 2012, Chen, Zhang et al. 2016, Wu, Han et al. 2018).

Table 2. Pseudo first and second order rate constants of resins calculated on the basis of total phenolics.

Model	**T (°C)**	**K_f**	**P**	**q_e**	**P**	**(R^2)**
Pseudo First order						
XAD 7HP	30	0.058	0.00	2.256	0.00	0.993
	40	0.063	0.00	2.036	0.00	0.994
	50	0.067	0.00	2.021	0.00	0.995
DAX 8	30	0.028	0.00	2.023	0.00	0.984
	40	0.029	0.00	1.853	0.00	0.990
	50	0.043	0.00	1.804	0.00	0.987
Model	**T (°C)**	**K_s**	**P**	**q_e**	**P**	**(R^2)**
Pseudo second order						
XAD 7HP	30	0.057	0.00	2.346	0.00	0.999
	40	0.073	0.00	2.108	0.00	0.998
	50	0.085	0.00	2.085	0.00	0.998
DAX 8	30	0.022	0.00	2.184	0.00	0.998
	40	0.025	0.00	1.991	0.00	0.999
	50	0.043	0.00	1.909	0.00	0.998

K_{f-} rate constant of pseudo first order; Ks- rate constant of pseudo second order; q_e-adsorption capacity at equilibrium, p-probability valor, R^2 - regression coefficient

3.3. Adsorption isotherm tests

Isotherm model was formulated to determine the maximum adsorption capacity (Lin and Juang 2009). According to static adsorption/ desorption tests and kinetics tests, DAX 8 and XAD 7HP showed the best results for adsorption Equilibrium, therefore, adsorption isotherms on XAD 7HP and DAX 8 resins at different temperatures of 30 °C, 40 °C and 50 °C for anthocyanins were obtained as shown in (Figure 4) and the total anthocyanin data (Table 3) were regressed according to the Langmuir, Freundlich and Henry isotherms equations.

Figure 4 shown that the adsorption capacities of anthocyanins increased with the initial concentration, similar results were reported by (Duran, Ozdes et al. 2011, Yang, Yuan et al. 2015, Nathália da R. Rodrigues, Junior et al. 2018). However, the adsorption capacity of TA was found to decrease following a rise in temperature from 30°C to 50°C for XAD 7HP and DAX 8 as shown in

figure 4 and value of predicted maximum adsorption capacity (Q_{max}) in table 3. Therefore, The results suggested that the adsorption processes of TA onto XAD-7HP and DAX 8 were exothermic in nature, which were in conformity with the results of the thermodynamic studies, similar results were obtained by (Jampani, Naik et al. 2014, Chen, Zhang et al. 2016).

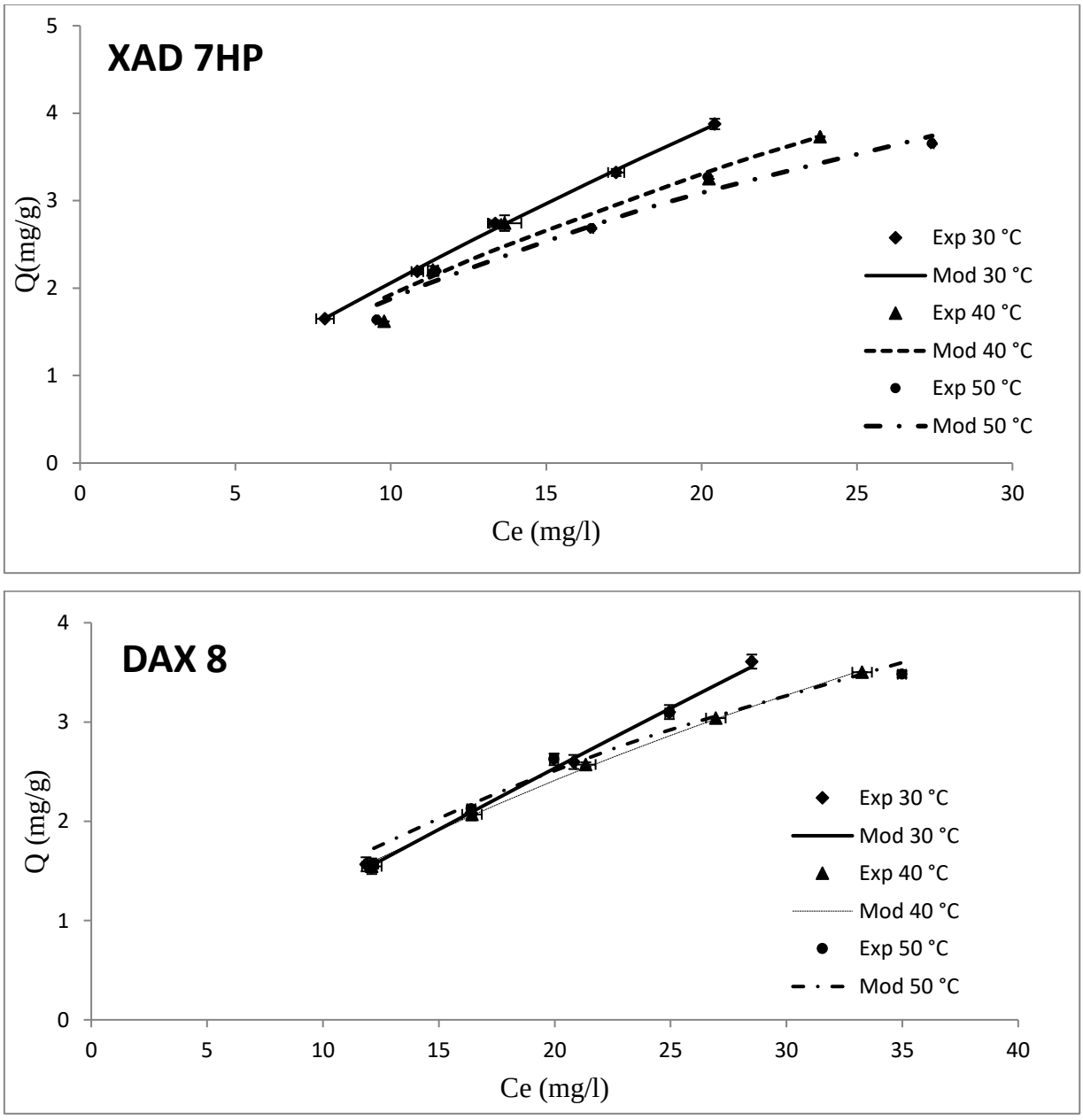

Figure 4. Adsorption isotherms of total antocyanin based on Langmuir equation on XAD 7HP and DAX 8 resins from GWPP extracts (Mod= model values, Exp= experimental values).

To establish the optimum model, the experimental equilibrium data were fit into three isotherm models: Langmuir, Freundlich and Henry models.

According to the correlative coefficients (R^2) showed in the (Table 3), data were well fitted by Freundlich and Langmuir model for both resins. The Langmuir and Freundlich isotherms are the most common models to express adsorption mechanism (Buran, Sandhu et al. 2014).

Langmuir isotherm model described the monolayer adsorption onto a homogeneous surface with no interaction between adjacent adsorbed molecules (Huang, Sun et al. 2011). The correlation

coefficients (R^2) of the Langmuir equation for TA on XAD-7HP were 0.9991, 0.9717 and 0.9849 at 30 °C, 40 °C and 50 °C respectively while the (R^2) values of the Langmuir equation on DAX 8 were 0.9986, 0.9992 and 0.9812 at 30 °C, 40 °C and 50 °C respectively, the (R^2) values of the Langmuir and Freundlich equation for both resins XAD 7HP and DAX 8 were more than Henry equation.

The high correlation coefficients indicated that the models were suitable to describe the tested adsorption system in the concentration range studied. K_L is the Langmuir constant, the values of K_L indicate whether the isotherm is favorable ($0 < K_L < 1$), linear ($K_L = 1$), or unfavorable ($K_L > 1$). The values of K_L at different temperatures summarized in Table 3 suggested that the isotherm of XAD-7HP and DAX 8 were favorable.

Compared with the Langmuir isotherm model, the Freundlich isotherm model reflects the adsorption process on a heterogeneous surface and is suitable to describe adsorption in a narrow range of solute concentration (Gao, Yu et al. 2013). K_F is a Freundlich affinity parameter for a hetero-disperse system, n is related to the magnitude, which is related to the sorption driving force and the energy distribution of the sorption sites, and the value of 1/n indicates the type of isotherm (Chen, Yang et al. 2014). Adsorption can take place easily when the value of 1/n is between 0.1 and 0.5, but when the value of 1/n is between 0.5 and 1; it is difficult for adsorption to occur. Furthermore, adsorption occurs with difficulty if the 1/n value exceeds 1 (Vasiliu, Bunia et al. 2011). As shown in the Table 3, the values of 1/n of Freundlich equation reflected that the adsorption of TA on XAD-7HP and DAX 8 were difficult to occur at the selected temperature, indicating that the adsorption of TA on XAD-7HP and DAX 8 resins couldn´t take place easily, overmore, the (R^2) values of the Langmuir equation on XAD 7HP and DAX 8 were better than Freundlich equation.

In this regard the results obtained from the Langmuir adsorption isotherm provided further confirmation of the exceptional promise in the separation of total anthocyanin for the both selected resins. The similar results were indicated by (Sandhu and Gu 2013, Jampani, Naik et al. 2014)

Table 3. Langmuir, Freundlich and Henry equation constants of total anthocyanin on Amberlite DAX 8 and XAD 7HP.

Model	**T ° C**		**K_l**	**p**	**Q_{max}**	**p**	**(R^2)**
Langmir							
XAD 7HP	30		0.0092	0.023	24.384	0.015	0.9991
	40		0.0197	0.207	11.673	0.119	0.9717
	50		0.02755	0.062	8.690	0.021	0.9849
DAX 8	30		0.0018	0.351	69.56	0.312	0.9986
	40		0.0132	0.003	11.529	0.001	0.9992
	50		0.02078	0.081	8.547	0.030	0.9812
Model	**T ° C**	**1/n**	**K_f**	**p value**		**p value**	**(R^2)**
Freundlich							
XAD 7HP	30	0.8864	0.26782	0.000838		0.00005	0.9988
	40	0.7664	0.33097	0.063612		0.00781	0.9681
	50	0.6817	0.395530	0.031554		0.00447	0.9783
DAX 8	30	0.6817	0.395530	0.031554		0.00447	0.9988
	40	0.7779	0.232291	0.002822		0.00018	0.9975
	50	0.6881	0.315021	0.054629		0.00659	0.9714
Model	**T ° C**		**K**	**p value**			**(R^2)**
Henry							
XAD 7HP	30		0.195518	0.000001			0.9921
	40		0.167843	0.000036			0.9276
	50		0.151856	0.000084			0.8826
DAX 8	30		0.125999	0.000000			0.9983
	40		0.113150	0.000009			0.9638
	50		0.114667	0.000085			0.8765

Q_{max} - maximum adsorption capacity, R^2 - regression coefficient, K_l - Langmuir constant., K_f - Freundlich constant, K- henry constant, n – adsorption intensity.

Finally, based on the results of static, kinetic and isotherm tests XAD 7HP showed higher adsorption capacity of TA (1.596.mg. g^{-1}) and higher recovery (81.7%), Therefore, XAD 7HP was selected as the best resin for further antioxidant tests and analysis profile of anthocyanin separated by it.

3.4. Antioxidant capacity of the initial and purified anthocyanin extract

The antioxidant capacity of GWPP extract and purified anthocyanin depends on their active composition and the conditions of the test system, which cannot be fully described by one single method due to many potential factors (Huang, Ou et al. 2005, Villano, Fernández-Pachón et al. 2006) .

Therefore, in this study, two methods (DPPH test and FRAP assay) were employed to evaluate the antioxidant capacities of GWPP and purified anthocyanin as shown in Table 4 and figure 5 respectively using external calibration and the results were expressed in Trolox equivalent (TE), according to results in Figure 5 and (Table 4) there was no significant difference ($p > 0.05$) in the antioxidant value of the two methods, similar results were reported by (Liu, Chang et al. 2016), however DPPH assay is widely used to establish antioxidant capacities in pure natural extract compounds for evaluation and selection.

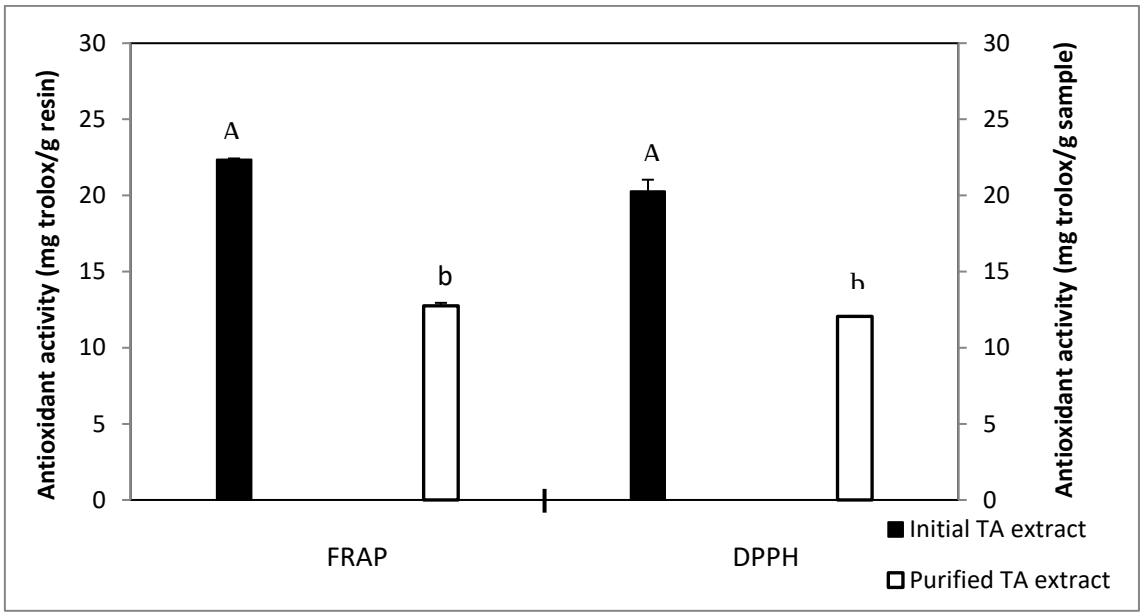

Figure 5. Antioxidant activity of initial and purified anthocyanin extract by XAD 7HP from GWPP. Results are mean of two determinations. Similar upper-case letter and similar lower- case letter indicate no significant differences between both methods of FRAP and DPPH ($p > 0.05$).

The results indicate that purified anthocyanin extract showed high antioxidant capacity in the both methods of FRAP and DPPH. Antioxidant capacity measured by DPPH was 20.29±0.75 (mg TE g^{-1}), 12.06 ± 0.019 (mg TE g^{-1}) in initial anthocyanin extract and purified anthocyanin extract respectively, these values are in agreement with (Beres, Simas-Tosin et al. 2016) who reported antioxidant capacity in a range of 23.03 ± 0.93 to 43.80 ± 4.90 mMol Trolox/100 g for grape pomace.

Table 4 Total anthocyanin contents TAC and antioxidant capacity in initial extract and purified extract of GWPP (n= 2).

Sample	**TAC (mg/100g)**	**Antioxidant Capacity**	
		DPPH (mg TE. g^{-1})	FRAP (mg TE. g^{-1})
Initial TA extract	230.381±1.899	20.29 ± 0.747A	22.35± 0.075A
Purified TA extract	193.702±0.0138	12.06 ± 0.019b	12.75 ± 0.192b

All the trials were performed in duplicate (n = 2) and all the data represent the means ± Standard deviation, data in the same row with similar letters is no significant difference ($p > 0.05$).

3.5. HPLC–PDA analysis

As shown in the results above XAD 7HP presented higher adsorption capacity of TA and higher recovery. Therefore it was used to separate anthocyanin compounds in order to understand the basic structure as well as the structural stability of anthocyanins, the chemical structures were determined by HPLC–PDA, and the results are shown in Figure. 6. The major anthocyanin peaks in the initial and purified extract were identified by comparing the retention times of the standards, the m/z of each anthocyanin molecule, and the fragmentation patterns with the previous value.(Barnes, Nguyen et al. 2009).

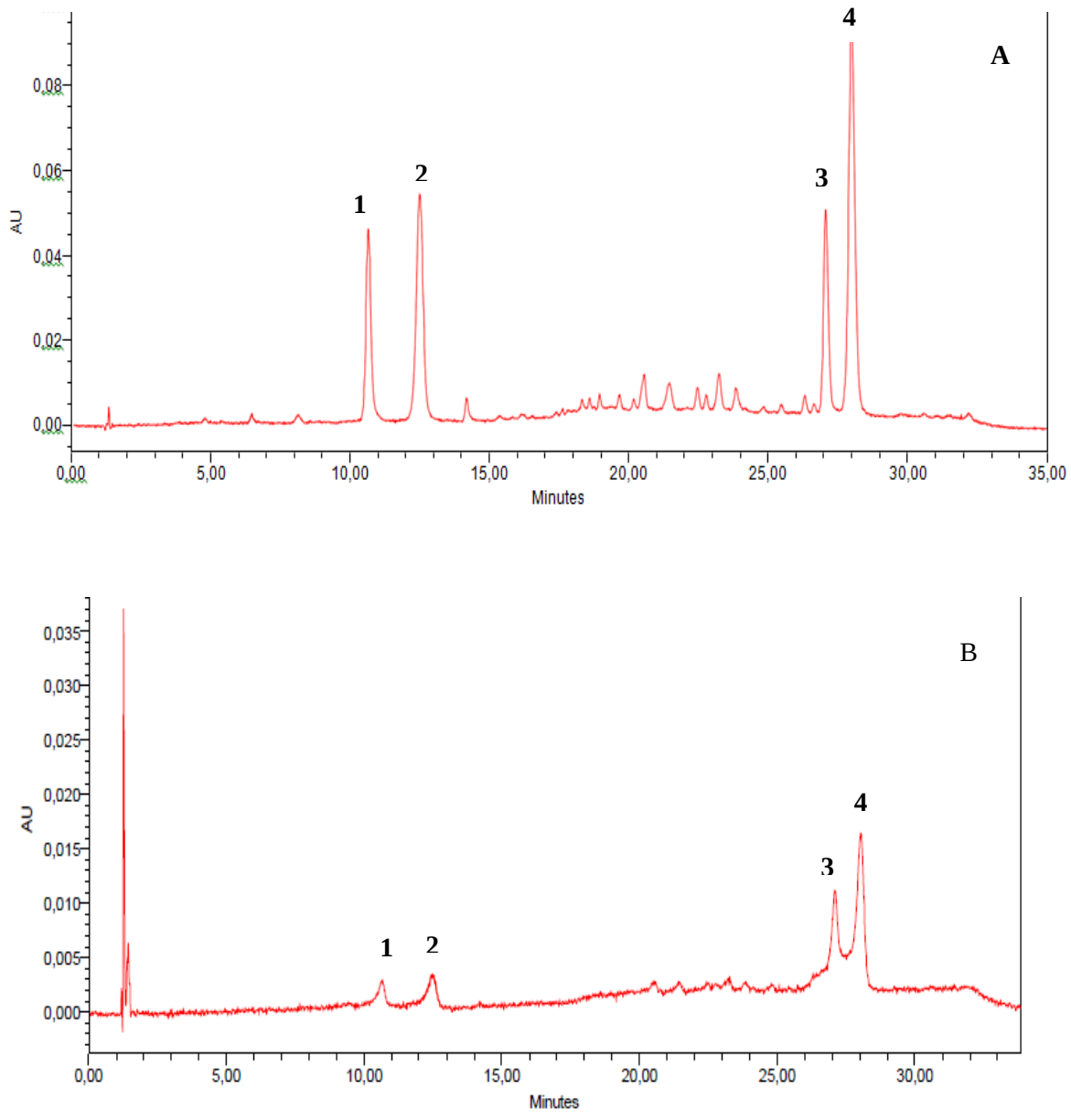

Figure. 6 High-performance liquid chromatography separation of anthocyanins from initial and purified extract of grape wine peel pomace A, B.

The structure of pigments at inicial and purified anthocyanin extract did not get affected during the process of separation. The chromatographic and spectroscopic characteristics, and composition of anthocyanins from GWPP obtained by HPLC– DAD–MS/MS are given in Table 5.

It can be observed from the table that seven different anthocyanins could be identified where Acylated Peonidin and Malvidine-3-O-glucoside are the two main components in GWPP and XAD 7HP resin showed recover percent of 63.64% and 59.533% for Malvidine-3-O-glucoside and Acylated Peonidin respectively, similar results were reported by (Kammerer, Kljusuric et al. 2005). Remarkably, the initial and purified extracts were rich in acylated anthocyanins (67.06%) and (71.33%) respectively, demonstrating that purified anthocyanin extract from pomace of *Alicante Bouschet* grapes to be a suitable source of natural colorants which could also be used for low-acid food applications, these results come to be consistent with (Kammerer, Kljusuric et al. 2005).

Table 5 Chromatographic and spectroscopic characteristics, and composition of anthocyanins from GWPP obtained by HPLC–PDA.

Peak	**Anthocyanin**	**t_r (min)**	**λ_{max} (nm)**	**$Area_I$ %**	**$Area_p$%**
1	Peonidin-3-O-glucoside	10,669	279.5, 520.1	15,97	8.83
2	Acylated Peonidin	12,507	280.7, 528.7	26,16	15.39
3	Malvidin-3-O-glucoside	27,085	275.9, 523.8	15,52	19.85
4	Acylated malvidin	28,008	280.7, 533.6	40,90	55.94

t_r – retention time.

$Area_I$% - quantification of the main anthocyanins in initial extract.

$Area_p$% - quantification of the main anthocyanins in purified extract.

4. Conclusion

In conclusion, this study has provided insights into the separation and purification of anthocyanins from grape wine peel pomace extract using seven kinds of macroporous resin including XAD 2, XAD 4, XAD-7HP, DAX 8, XAD 11 and XAD 16 where XAD-7HP showed higher adsorption capacity of TA (1.596.mg. g^{-1}) and higher recovery (81.7%) which can be attributed to its similarity to the polarity of anthocyanin. The pseudo second-order model which is considered better to predicts the behaviour over the whole range of adsorption had confirmed a better capacity than pseudo first-order model to predict adsorption capacity of anthocyanins from GWPP extract, furthermore langmuir adsorption isotherm provided further confirmation of the exceptional promise in the separation of total anthocyanin for the both selected resins XAD-7HP and DAX 8 with suggestion of isothermic status for adsorption process of TA regarding to the results of Adsorption isotherm tests. Moreover, antioxidant capacity of initial TA extract and purified TA has been tested and the results showed that purified TA extract showed high antioxidant capacity in the both methods of FRAP and DPPH where the pyrone ring and the number of free hydroxyls around play a significant role in antioxidant activity of anthocyanins. Regarding to high-performance liquid chromatography analysis, acylated Peonidin, Peonidin-3-glucoside, acylated malvidin and malvidine-3-glucoside are the four main anthocyanin compounds in the initial and purified extract of grape wine peel pomace *Alicante Bouschet*. This purified anthocyanin extract from GWPP could potentially find application as a natural colorant, dietary supplement, antioxidant ingredient for functional foods, and/or as a raw material in the cosmetic and pharmaceutical industry preparations.

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Conclusão Geral

A antocianina é um composto importante e influente na saúde humana, além de algumas aplicações no campo da indústria de alimentos. No entanto, o mecanismo para o impacto preciso da antocianina no corpo humano não foi identificado até agora e a pesquisa ainda continua a descobrir mais compostos de antocianinas encontrados em diferentes variedades de plantas.

Dentre os 6 diferentes tipos de resinas estudadas, as resinas DAX 8 e XAD 7HP demonstraram-se mais eficientes na obtenção de extratos purificados de antocianinas provenientes resíduo da fermentação de casca da uva *Yitis vinifera L*. var., o que foi definido pelos parâmetros de maior capacidade de adsorção e dessorção e maior percentual de recuperação durante os testes estáticos. As diferenças de comportamento entre as resinas podem ser explicadas devido às distintas propriedades físicas e afinidade que as mesmas apresentam em relação ao composto de interesse estudado.

Os testes de adsorção cinética demonstraram que a resina XAD 7HP permite maior capacidade de adsorção das antocianinas em relação à resina DAX 8, embora apresente um tempo semelhante para atingir o equilíbrio de adsorção, além disso, o modelo de pseudo-segunda ordem do teste de cinética de adsorção confirmou uma melhor capacidade do que o modelo de pseudo-primeira ordem para prever a capacidade de adsorção de antocianinas do extrato de GWPP.

O estado isotérmico para o processo de adsorção de AT foi sugerido em relação aos resultados dos testes de isotermas de adsorção. Ademais, a isoterma de adsorção de langmuir forneceu uma confirmação adicional da promessa excepcional na separação da antocianina total para ambas as resinas selecionadas XAD-7HP e DAX 8.

Peonidina Acilada, Peonidina-3-Glicosídeo, Malvidina Acilada e Malvidina-3-Glicosídeo são os quatro principais compostos de antocianina no extrato inicial e purificados de grape wine peel pomace *Alicante Bouschet* em baseado de cromatografia líquida de alta eficiência. Além disso, o extrato AT inicial purificado mostrou alta capacidade antioxidante nos dois métodos de FRAP e DPPH.

Este extrato de antocianina purificado de GWPP poderia potencialmente encontrar aplicação como um corante natural em diferentes tipos de alimentos acidificados e adequados para colorir os produtos lácteos, como creme de leite e iogurte, que têm níveis de pH em torno de 4,2 - 4,5., suplemento dietético, ingrediente antioxidante para alimentos funcionais, e / ou como matéria-prima nas preparações da indústria cosmética e farmacêutica.

ANEXOS

1. Determination of Total anthcyanins Content

1.1. Preparation of solutions

1.1.1. Buffer solution pH 1.0

3.725 g of potassium chloride was dissolved in 250 mL of distilled water (Solution A). 13.10 mL of concentrated hydrochloric acid was diluted to 770 mL of distilled water (Solution B). Slowly solution (B) was added to solution (A), the pH was measured at each moment and adjusted to pH = 1 with solution (B). the solution was stored in a covered bottle.

1.1.2. Buffer solution pH 4.5

54.40 g of sodium acetate was dissolved in 400 mL of distilled water (Solution C). 19.90 mL of concentrated hydrochloric acid was diluted to 240 mL of distilled water (Solution D). Slowly solution (D) was added to the solution (C), the pH was measured at each moment and adjusted to pH = 4.5 with solution (D).

1.2. Preparation of Test Solution

The maximum test portion added should be (1 part test portion, 4 parts buffer) so as not to exceed the buffer capacity of the reagents.
The appropriate dilution factor was determined by diluting the test portion with pH 1.0 buffer until absorbance at 520 nm is within the linear range of the spectrophotometer. (For most spectrophotometers, the absorbance should be between 0.2 and 1.4 AU.) Using this dilution factor, 2 dilutions of the test sample were prepared, one with pH 1.0 buffer and the other with pH 4.5 buffer.

2. FRAP Tests

The results were expressed in Trolox equivalent using Trolox antioxidant standards: FRAP/ TROLOX : $y = 15.772\ x - 0.0025$; $R^2 = 0.9939$; x: 0,0059 – 0.0539 µM of TE; n= 8).
(TE) Trolox Equivalent by µmol, n is the number of different concentration, (x, in µM of each antioxidant standard) used in the construction of the calibration curves.

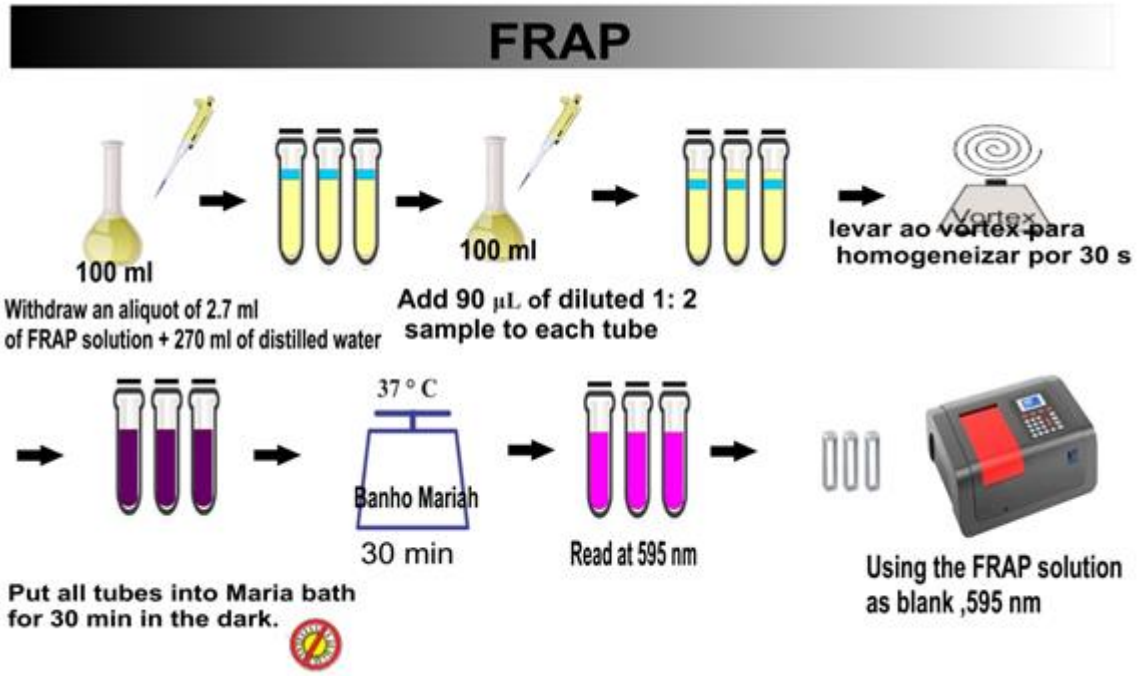

Figura 3. FRAP Test

3. DPPH Tests

The results were expressed in Trolox equivalent using Trolox antioxidant standards:DPPH/ TROLOX :

$y = 14.252\ x + 0.7258$; $R^2 = 0.9954$; x: 0.0059 – 0.0419 µM of TE; n=7).

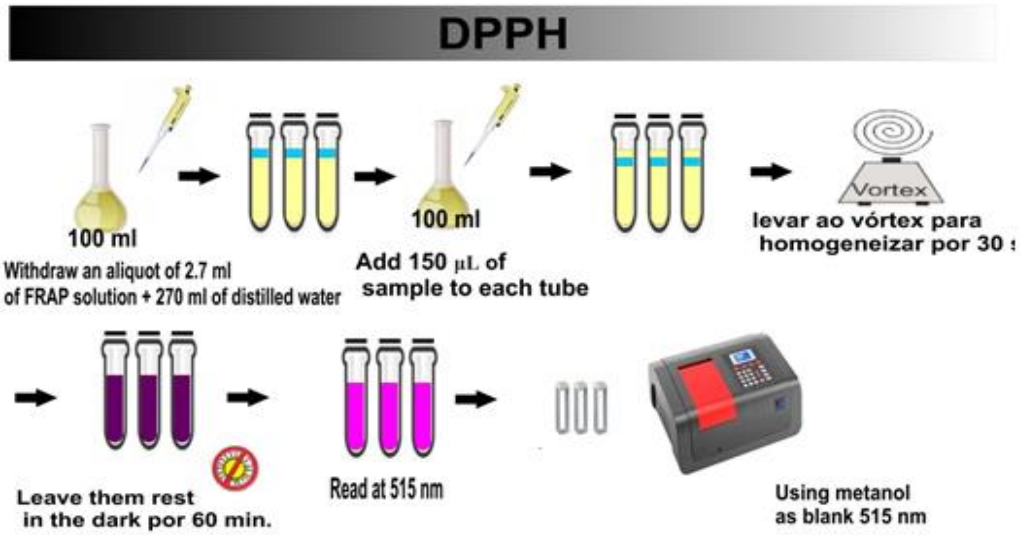

Figure 4.DPPH Test