

1-1-2014

Comparative Phenolic Content And Antioxidant Activities Of Blueberry (*vaccinium Corymbosum*) Afected By In Vitro Digestion

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**COMPARATIVE PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF
BLUEBERRY (*VACCINIUM CORYMBOSUM*) AFFECTED BY IN VITRO DIGESTION**

by

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THESIS

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

2014

MAJOR: NUTRITION AND FOOD SCIENCE

Approved by:

Advisor

Date

DEDICATION

This thesis is dedicated to my parents for their endless love, support and encouragement. To my husband who gives me confidence and patience. My achievement is also dedicated to my daughter who taught me the meaning of love. A special feeling of gratitude to my loving sisters and brother whose words of encouragement and push for tenacity ring in my ears. My in-laws have never left my side and are very special, I will always appreciate all they have done.

ACKNOWLEDGEMENTS

I would never have been able to finish my thesis without the guidance of my committee members, help from friends, and support from my family and husband.

I would like to express my deepest gratitude to my advisor, Dr. Kequan Zhou, for his excellent guidance, caring, patience, and providing me with an excellent atmosphere for doing research. Special thanks goes to Dr. Ahmad Heydari and Dr. Yifan Zhang, who were willing to participate in my final defense committee at the last moment.

I would like to thank Dr. Arvind Goja who helped me during my experiments. I would also like to thank Houda, who as a good friend, was always willing to help and give her best suggestions. It would have been a lonely lab without her. Many thanks to Wenjun patiently corrected my writing and giving me advises.

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INTRODUCTION

Oxidative Stress and Health

Reactive oxygen species (ROS) are constantly produced in cells by cellular metabolism and by exogenous agents. They are essential for life because they are involved in cell signaling and are used by phagocytes for bactericidal action [1,2]. In a biological field, reactants and oxidants are called antioxidants and pro-oxidants. Pro-oxidants are separated into two groups of species: reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3,4]. The oxygen derived molecules are O^{2-} (superoxide), $HO\cdot$ (hydroxyl), HO_2 (hydroperoxyl), $ROO\cdot$ (peroxyl), $RO\cdot$ (alkoxyl) as free radicals and H_2O_2 oxygen as non-radical. Nitrogen derived oxidant species are mainly NO^- (nitric oxide), $ONOO^-$ (peroxy nitrate), NO_2 (nitrogen dioxide) and N_2O_3 (dinitrogen trioxide) [5,6,7].

Recently, increasing evidence highlights that overproduction of ROS and oxygen-derived free radicals may contribute to a variety of pathological effects, for example, DNA damage, carcinogenesis and cellular degeneration [8,9].

Berries contain high concentration in bioactive compounds such as polyphenols, including anthocyanin, phenolic acids, tannins, carotenoids, vitamin A, C, E, folic acid and minerals such as calcium, selenium and zinc [10,11]. Among them, blueberries became well known and often consumed due to their uses for treating biliary disorders, coughs, tuberculosis, diabetes and visual disorders [12,13,14]. Blueberries contain high level of anthocyanin and phenolic compounds with high in vitro antioxidant capacities compared with other fruits [15,16].

The main characteristic of antioxidants is their ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals can oxidize nucleic acids, proteins, lipids or DNA and initiate

degenerative diseases [17,18]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases [19].

Oxidative stress is an important factor in ischemic stroke, which occurs as a result of an obstruction with a blood vessel supplying blood to the brain [20]. Results from a study conducted by Sweeney et al. indicated that adding blueberries to the diet of rats could reduce the effects of ischemic stroke by half [21].

Joseph et al. studied the effect of a diet high in fruit and vegetable extracts on rats [22]. The team determined that a diet supplemented with extracts from strawberries, blueberries, or spinach improved motor skills and short-term memory loss. Further studies suggested that berry supplementation could overcome the genetic predisposition to Alzheimer's disease [23]. The anti-inflammatory potential of the polyphenols in blueberries, including the potent antioxidant anthocyanin, was the focus of a 2008 study [24]. When rats with neuronal lesions were fed a blueberry-supplemented diet, not only did they perform better in cognitive tests, the concentration of several substances in the brain that can trigger an inflammatory response was significantly reduced [25, 26]. The polyphenols in blueberries appeared to inhibit the production of these inflammatory mediators.

Antioxidants and Their Mode of Action

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the cell components by neutralizing the damaging effects of free radicals, which are natural by-products of cell metabolism [3].

The oxidative stress induced by reactive oxygen species (ROS) can be described as a dynamic imbalance between the amounts of free radicals generated in the body and levels of

antioxidants to quench and/or scavenge them and protect the body against their deleterious effects [5]. The first strategy of defense against ROS is preventing mechanisms of radical formation [25]. Carotenoids, a class of plant pigments, are well-known compounds that can quench electronically excited molecules, such as singlet molecular oxygen, thus preventing oxidative damage [4]. Grapes contain a natural phytoalexin, resveratrol, which can inhibit ribonucleotide reductases known to generate radical substrates [6]. Preventing the initiation of chain reactions through binding metal ions is also important in preventing radical formation, especially chelating iron and copper ions, a function performed by a variety of natural antioxidants.

In a normal cell, there is appropriate oxidant: antioxidant balance. However, this balance can be shifted, when production of oxygen species is increased or when levels of antioxidants are diminished. This stage is called oxidative stress [7].

An antioxidant defense system is used by the human body to neutralize the excessive levels of reactive oxygen species [26]. This system is composed of enzymatic and non-enzymatic antioxidants [26]. Some of the antioxidant enzymes that are found to provide a protection against ROS are superoxide dismutases, catalases, and glutathione peroxidases, in addition to numerous non-enzymatic small molecules distributed widely in the biological system and capable of scavenging free radicals [27,28]. These non-enzymatic molecules include glutathione, tocopherol (vitamin E), vitamin C, β -carotene, and selenium [28].

Determining Bioaccessibility of Antioxidants by *in vitro* Digestion

Simulating gastric and intestinal digestion for the approach of *in vitro* digestion of food have been used to investigate digestion of proteins, starch, lipids, polyphenols, and carotenoids from various matrices [29, 30, 31]. Digestive enzymes including pepsin and pancreatin along

with bile salts, time, pH, and temperature can be controlled for a simulated digestion procedure [32]. These simulated digestion procedures give information about the stability of compounds during gastrointestinal digestion. An *in vitro* gastrointestinal system has been used to study the bioaccessibility of polyphenols and carotenoids in different food sources such as grape seed and peel, green tea, carrot, tomatoes, and leafy vegetables [33,34,35].

Previous Studies on Blueberry Antioxidants

Currently, plant sourced antioxidant agents have been attracting special interest because they can protect the human body from diseases induced by free radicals[36].

Vaccinium corymbosum are flowering plants belonging to the large genus of *Vaccinium*. Various members of the *Vaccinium* genus have been used as traditional medicines for the treatment of diabetic symptoms by Quebec traditional practitioners [37,38]. Blueberry leaves are primary plant parts that have been used medicinally for generations[39].

Blueberries (*Vaccinium sp.*) are a good source of chlorogenic acid, quercetin, kaempferol, myricetin, procyanidins, catechin, epicatechin, resveratrol, and vitamin C which participate to their antioxidant activity[40]. Blueberries (*Vaccinium sp.*) are rich in anthocyanins, polyphenolics recognized for their ability to provide and activate cellular antioxidant protection, inhibit inflammatory gene expression, and consequently protect against oxidant-induced and inflammatory cell damage and cytotoxicity[41,42].

Research has shown that wild blueberries (*Vaccinium angustifolium*) are one of the highest sources of anthocyanins [43, 44] and have exhibited one of the highest recorded *in vitro* antioxidant capacities of various fruits and vegetables tested [45]. Wild blueberries are relatively low in antioxidant vitamins and minerals [46]. Their *in vitro* antioxidant capacity has been attributed to their high concentration of phenolic compounds, particularly anthocyanins [45,46].

More recently, Mazza et al. investigated the absorption of anthocyanins in humans after the consumption of a high-fat meal with a freeze-dried blueberry powder containing 25 anthocyanins [47]. 19 anthocyanins present in the blueberries were detected in human blood serum. Furthermore, the appearance of total anthocyanins in the serum was directly correlated with an increase in serum antioxidant capacity[47].

In recent years, researchers have developed concerns regarding antioxidant bioavailability after gastrointestinal digestion. Bioactive compounds should be bioavailable in order to perform the desired functions. These compounds are usually subject to series of physiological processes such as absorption, metabolism, tissue and organ distribution, and excretion [36]. For these reasons, recent research has focused on the stability of these compounds against these processes.

Several studies have examined the bioaccessibility of these compounds after *in vitro* digestion. For example, commercial fruit juices, which are well-known easy-to-get antioxidant sources, were analyzed for antioxidant stability after *in vitro* digestion. The results show that gastrointestinal digestion seems to enhance antioxidant activity for some of them [65]. In a different study, blackberry polyphenols were tested before and after digestion and the latter is efficient in enhancing intracellular capacity [54]. Total polyphenols could be sensitive to simulated digestion conditions and some may be modified into favorable health-promoting structures [55,62].

OBJECTIVE

The objective of this study was to investigate the effect of in vitro gastrointestinal digestion on the antioxidant content and capacity of *Vaccinium Corymbosum* species. Total phenolic content (TPC) assay, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and oxygen radical absorbance capacity (ORAC) assay were used to estimate the antioxidant characteristics of the sample with active and inactive enzymatic digestion. Also to study the antioxidant methods applied in relation to the total phenolic content.

MATERIALS AND METHODS

Reagents

Pancreatin, pepsin, Gallic acid, Follin-ciocalteu's phenol reagent and sodium carbonate (Na_2CO_3) were purchased from Sigma-Aldrich (St. Louis, MO). Acetone, methanol, and formic acid were obtained from Fisher (Fair Lawn, NJ). Bile salts, hydrochloric acid (HCl) and fluorescein were acquired from Fluka (Buchs, Switzerland). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Aldrich (Milwaukee, WI). Trolox was purchased from ACROS (Geel, Belgium). Ethanol and Acetone were acquired from Decon labs (King of Prussia, PA). Sodium hydroxide was obtained from Mallinckrodt (Phillipsburg, NJ). 2, 2'-Azobis (2-midinopropane) dihydrochloride (AAPH) was purchased from Wako (Richmond, VA).

Samples and standard preparation

958.74 g of fresh blueberry (*Vaccinium Corymbosum*) was purchased from a local grocery store in Michigan; the sample was stored at -20°C immediately upon arrival. Frozen samples were chopped into small pieces in order to create a larger surface area for freeze-drying. They were then stored overnight at -80°C prior to freeze-drying. Following freeze-drying using Labconco lyophilizer Lyph Lock 6 (Kansas City, MO), dried fruits were milled into fine powder, which was further divided into two groups (Control and Treatment).

The two groups labeled Control (C) and Treatment (T) were used for *in vitro* digestion study, after which antioxidant activity assays were performed. The first group was subject to treatment of simulated digestion with inactive enzymes whereas the second group was subject to digestion treatment with active enzymes.

In vitro Digestion

The bioavailability of antioxidant compounds from blueberries was assessed using an *in vitro* digestion procedure that mimics the physiochemical and biochemical changes that occur in the upper gastrointestinal tract.

A shaking water bath (Pegasus scientific, Rockville, MD) was used to perform the *in vitro* digestion. The method consisted of two sequential steps: an initial pepsin/HCl digestion for 2 h at 37 °C to simulate gastric conditions followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate small intestine conditions. The first step consisted of dissolving 1 g of pepsin in 100 mL distilled water to make the pepsin solution, at the same time 0.4 g of pancreatin and 2.5 g of bile salts were dissolved in 100 mL distilled water to make the pancreatin-bile solution. Control and treatment were treated with these solutions. For the control group, the solutions were boiled then cooled down to room temperature before treatment in order to inactivate the digestive enzymes. The procedure (for both T and C groups) was as follows: 1 g of dried fruit powder was mixed with 2 mL pepsin solution (inactive for C, active for T) and 17 mL distilled water. Then the pH was adjusted to 1.7 - 2 by the addition of concentrated HCl, and the samples were incubated in a 37 °C shaking water bath at 100 rpm for 2 h. Following the pepsin digestion to simulate small intestine conditions, reaction mixtures were adjusted to pH 8.0 with 1N NaOH and added with 2 mL of pancreatin-bile salts solution (inactive for C; active for T). The mixtures were incubated in the water bath shaker for 2 hours at 100rpm. Following the simulated digestion process, solutions were stored at -80°C overnight then freeze-dried. All dried samples were then extracted with 50% acetone and vortexed 3 times for 5 minutes each. The extracts were filtered through a 0.45 µm filter paper and diluted with 50% acetone to a concentration of 50 mg/mL. These samples were then used for antioxidant assays: total phenolic

content (TPC) assay, DPPH radical scavenging assay, and oxygen radical absorbance capacity assay (ORAC).

Total Phenolic Content (TPC) Assay

The amount of total polyphenol in the blueberry extracts was determined using modified Folin-Ciocalteu colorimetric method. Stock solution of sample extracts were dissolved in acetone to make 25mg/mL and further serial dilutions were performed to obtain readings within the standard curve made with Gallic acid. The standard curve has a concentration of Gallic acid as follow: 0.1, .02, 0.3, 0.4, 0.5 mg/mL in 50% acetone. The measurements were done in triplicate and results were expressed as the mean values. Each test tube contained 25 μ L of sample/standard and 250 μ L distilled water. 750 μ L of 0.2 N Folin-Ciocalteu's phenol reagent was then added to each tube and mixed thoroughly with a vortex mixer. Then, 500 μ L of 20% sodium carbonate was added to each tube and vortexed again. After that all samples and standards were incubated in the dark for 2 hours at room temperature. The extracts were oxidized by the Folin-Ciocalteu reagent and the neutralization was made with Na₂CO₃, after 5 minutes. Detection was achieved at 765 nm in a UV spectrophotometer Total phenolic content assay was tested with a Beckman DU 640 spectrophotometer and results were expressed as milligrams of Gallic acid equivalents (GAE) per 100 grams fresh fruit weight.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH scavenging activity assay was done according to a method reported by Brand-Williams *et al.* with modification [48]. This assay measures the ability of blueberry extracts to reduce DPPH free radicals. DPPH solution (80 μ M) was freshly prepared by dissolving the reagent in 50% acetone. Sediment-free sample solutions were diluted to 10 mg/ml using 50% acetone and then collect from the supernatant after centrifuged at 7200 rpm for 20 min. A

volume of 150 μL of this solution was allowed to react with 100 μL sample in a 96-well microplate, and the absorbance was measured at 500 nm every 5 minutes for 2 hours using the bio assay reader (HTC 7000 Bio Assay Reader (Perkin Elmer, Norwalk, CT) . All samples were run in duplicates. The chemical kinetics of blueberries extract was recorded. The antioxidant activity was calculated as follows:

$$\% \text{ DPPH scavenging activity} = (\text{Abs control} - [\text{Abs sample} - \text{Abs sample background}] / \text{Abs control} \times 100$$

Oxygen Radicals Absorbance Capacity (ORAC_{FL}) Assay

The protocol followed by Zhou et al. was used to measure the Oxygen Radicals Absorbance Capacity of the blueberries sample [49]. Briefly, AAPH was dissolved in 75 mM phosphate buffer (pH 7.4) to a final concentration of 0.36 M and made fresh daily. A fluorescein stock solution (10000 nM) was made in 75 mM phosphate buffer (pH 7.4) and stored wrapped in foil at 5°C. Immediately prior to use, the stock solution was diluted to 8000 nM with 75 mM phosphate buffer (pH 7.4).

Serial dilutions of standard (Trolox) were performed to achieve concentrations of 0, 20, 40, 80, 100, 200, 300 and 400 μM with 50% acetone. Control samples were diluted to a concentration of 1.3 mg/mL with 50% acetone. 200 μL of fluorescein and 45 μL of samples or standards were placed in each well of a 96-well microplate. The microplate was incubated for 15 minutes at 37°C. 60 μL of AAPH was then added to each sample and standard; and fluorescence was measured at 37 °C in plate reader (HTC 7000 Bio Assay Reader (Perkin Elmer, Norwalk, CT). Fluorescence was then monitored kinetically with data taken every 5 minutes for 90 minutes using an excitation wavelength of 485 nm and an emission wavelength of 520 nm. All samples and standards absorbance were measured in triplicates. Radical absorption capacity was

calculated using a standard curve established with various concentrations of Trolox. Results are presented as mM Trolox equivalent (TE)/ 100 grams fresh weight.

Statistical Analysis

Antioxidant data executed from the above performed assays was analyzed via Microsoft Excel using independent t-test. Mean and standard deviation (SD) were calculated for each parameter. Data is therefore reported as mean \pm SD. Outcomes were compared using $p \leq 0.05$ as a cutoff point for statistical significance.

RESULTS

Total Phenolic Content (TPC) Assay

The antioxidant capacity of Blueberries was tested with TPC assay. The comparative data for total phenolic content of blueberry (C and T) is presented in **Figure 1**. The total polyphenols content was determined using the Folin-Ciocalteu method. Gallic acid was used as calibration standard and the results (expressed as Gallic acid equivalents) were expressed as means \pm standard deviation of triplicate analysis. The TPC value in the blueberry extract treated with deactivated enzymes was 8.35 g GAE/ 100 g fresh blueberry which showed significant difference from 11.29 g GAE/ 100g fresh weight in sample treated with active enzymes. Furthermore, the comparison of total phenolic content between C and T showed higher phenolic content in Treatment sample ($p < 0.001$).

DPPH Radical Scavenging Assay

Antioxidant capacity was then measured by DPPH radical scavenging assay which detects percent DPPH radical inhibition by antioxidants from blueberry. The DPPH scavenging activity of blueberries extract is presented in **Figure 2**. It has been obtained significant difference ($p < 0.05$) between the blueberries extracts analyzed, with a higher value for the treatment sample (35.14%) compared to control sample (27.93%).

Oxygen Radical Absorbance Capacity (ORAC) Assay

ORAC assay is widely used for the detection of free-radical scavenging ability of antioxidant against peroxy radical. Trolox was used as standard and test sample results were expressed as micromoles of Trolox equivalent per 100 gram fresh weight of blueberry. **Figure 3** shows a higher peroxy radical scavenging capacities 3320.52 $\mu\text{mol TE/ 100 g}$ for the sample treated with active digestive enzymes, whereas the sample treated with inactive digestive

enzymes presented ORAC value of 1829.95 $\mu\text{mol TE}/ 100\text{g}$ and found to display significantly different peroxy radical scavenging abilities ($p < 0.05$).

DISCUSSION

Blueberries are of interest in health and nutritional science because of their high phenolic content compared to other fruit crops [50]. The present study investigated antioxidant content and activities of *Vaccinium Corumbosum* blueberry.

It is widely believed that the antioxidant activity of plant phenolic (e.g., flavonoids, tannins, phenolic acids, etc.) resides mainly in their ability to donate hydrogen atoms or electrons and there by scavenge free radicals [51]. Plant phenolic compounds are nowadays getting increased attention in the diet due to their natural antioxidant potential. Increased consumption of phenolic compounds has been associated with the reduced risk of cardiovascular diseases and certain cancers [52, 53]. The mean value of total phenolic content of *Vaccinium Corymbosum* blueberry extracted using enzymatic approaches is shown in Figure 1. Therefore, according to our TPC results, active enzyme digestion seems to enhance antioxidant content. Figure 1 clearly reveals that the sample treated with active enzymes (T) has higher total phenolic contents than their inactive enzyme treated counterparts (C). These results indicate that the process of enzymatic digestion does not seem to destroy or reduce the antioxidant content of blueberry. Instead the antioxidant capacity of the intact fruit is preserved and even enhanced. Other studies with different fruits have shown varying results when comparing digested and undigested samples [54, 55, 56]. We can conclude from our results that *Vaccinium Corymbosum* blueberry has a higher concentration of antioxidants when digested.

The DPPH assay is simple, quick and commonly used to assess the antioxidant activity of plants and natural compounds, which act as free radical-scavengers or hydrogen donors in vitro [57]. Digested blueberry extract demonstrated appreciable scavenging properties against DPPH radicals. DPPH scavenging activity was significantly ($P < 0.05$) for sample treated with inactive

enzymes than its digested counterpart. Adding an antioxidant-rich sample to a DPPH solution causes a gradual reduction in absorbance which implies that DPPH radicals are being scavenged.

Therefore, the percentages we have presented pertain to DPPH radical scavenging capacity which in turn is directly proportional to antioxidant capacity. Our results indicate that in the concentration of 10 mg/mL, our digested sample (T) showed higher scavenging ability (35.14%) compared to control sample (27.93%) and therefore higher antioxidant capacity, which further reinforces TPC results discussed above. These findings also may indicate that the higher phenolic content that the digested samples are found to have may be correlated to their higher potency in scavenging DPPH radical of digested and undigested variants of fruits [56, 58]. Our results therefore present a comparison of such capacity in digested versus undigested blueberry that, to our knowledge, has not been reported before.

The ORAC assay which differs from DPPH radical scavenging assay by the type of radical produced, scavenging method and measurement, also showed results that support our TPC and DPPH results. AAPH produces peroxy radicals that are to be scavenged by antioxidants found in our fruit sample [59]. A molecular probe, fluorescein, is under peroxy radical attack. The more antioxidants, the more peroxy radicals being scavenged and the less fluorescence is detected (fluorescein is protected by antioxidants from radical attack). Higher Trolox equivalents calculated from a sample shows better ability of scavenging AAPH radicals. The digested blueberry sample shows a higher peroxy radical scavenging capacity than the undigested one, which further confirms the previously discussed results (TPC and DPPH). Several studies support the higher availability of antioxidants in a digested fruit when comparing to the raw or undigested fruit [60].

Blueberries as a natural source of antioxidants

Digested blueberry provides potentially higher antioxidant bioaccessibility that may have significant health benefits, when compared to their undigested counterpart. The stability of antioxidant capacity following gastrointestinal digestion has been analyzed in other berries [61, 62]. For instance, antioxidant capacity of chokeberries, strawberries, cranberries and blackberries was enhanced following in vitro digestion [63]. These findings support our findings that the digestion of some berries can enhance their antioxidant characteristics thus contributing to considerable health benefits in humans [64]. However, limited researches have been studies on blueberries with antioxidants activities after digestion when compared to other berries [64, 65].

A comparative study of TPC content of wild and cultivated blueberries has shown that the wild blueberry presented higher antioxidant content than the cultivated [66]. TPC content for cultivated Romanian blueberries shows that the total polyphenol content ranged from 4.25 – 8.19 g GAE/ g fresh weight which support our results that the digestion enhances the antioxidant content of the blueberry [67].

In the present study blueberry seems to have a higher DPPH when digested compared to other studies that reported a lower DPPH for raw wild and cultivated blueberries [38, 43]. In addition, a screening of ORAC in a variety of berries shows lower Trolox equivalents in 100 g of fresh weight when comparing to blueberry [67].

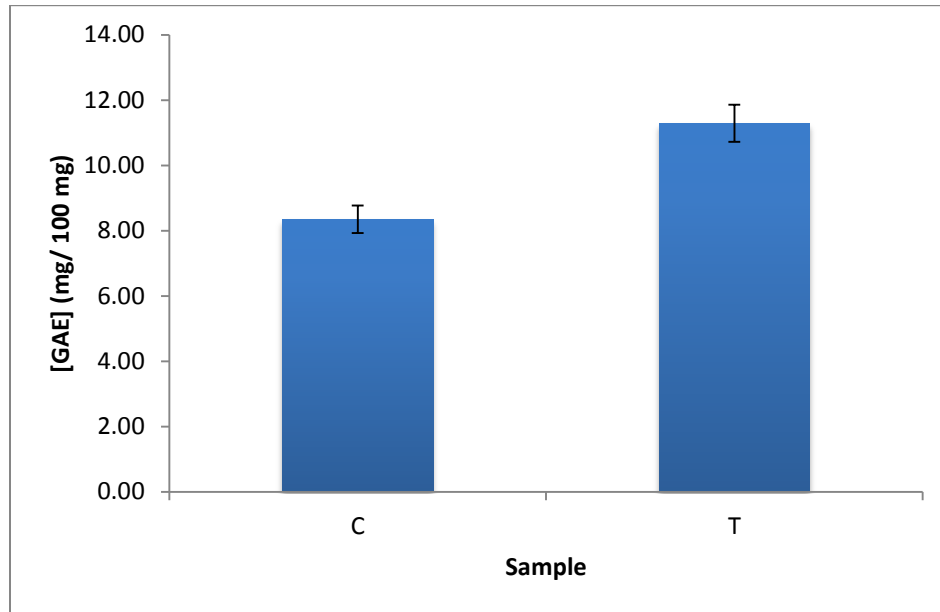
It seems that in the wide variety of fruit crops available, those with deeper colors are richer in antioxidant which is the case for blueberries [68]. Research supports deep colored berries as potent antioxidant sources [69]. Such berries are also rich in anthocyanin, antioxidant compounds that are known for their enhanced stability and bioaccessibility following gastrointestinal digestion [70].

In agreement with the above mentioned studies, we report that blueberry is a remarkable source of antioxidant compounds when compared to other berries. Our results imply that the blueberry has a significantly higher antioxidant capacity when digested. Also we can confidently state that digestion seems to enhance the antioxidant content and activity in blueberry.

CONCLUSION

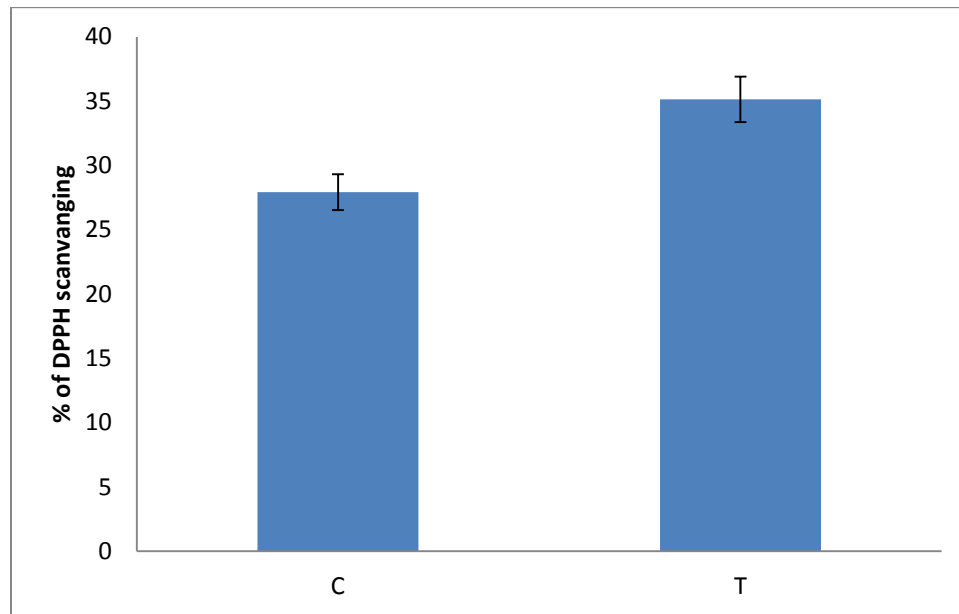
We can conclude that our results are quite valuable, due to the potential importance of the studied compounds in human's health and the relatively high amount present in blueberries fruits. The preliminary in vitro simulated digestion showed enhanced antioxidant capacity when blueberry subjected to such treatment. This indicated that enzymatic treatment similar to digestion would not decrease antioxidant value, which helps contribute to possible increased bioavailability. Our results suggest that the gastro-intestinal tract may act as an extractor where polyphenols are progressively released from solid matrix and made available for the absorption or to exert their biological effects in the gastro-intestinal tract. Further experimentation such as in vivo digestion model maybe performed in order to investigate more close correlation to human/mammal physiology. Also more research can be carried out to analyze the effect of each individual antioxidant compound from blueberry in gastrointestinal digestion.

Figure 1 - Total phenolic content of *Vaccinium Corymbosum* digested by inactive (C) and active digestive enzymes (T) (GAE g / 100 g fresh weight).



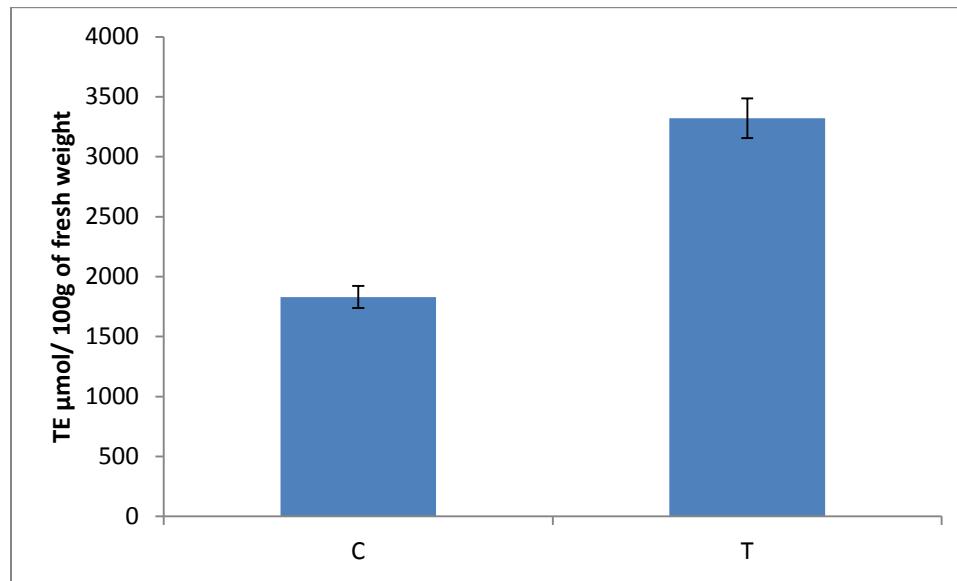
A significant difference between C and T groups presented higher phenolic contents in the T group ($p < 0.001$).

Figure 2 - DPPH radical scavenged in percentage of *Vaccinium Corymbosum* blueberry digested by inactive (C) and active (T) digestive enzymes.



A significant difference in DPPH % between C and T groups with a higher value for T group ($p < 0.05$).

Figure 3 - ORAC assay in TE ($\mu\text{mol}/100\text{g}$ fresh weight) of *Vaccinium Corymbosum* Blueberry digested by inactive (C) and active (T) digestive enzymes.



There is a significant difference between C and T groups and presented higher Trolox equivalent in the T group ($p < 0.05$)

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ABSTRACT**COMPARATIVE PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF BLUEBERRY (*VACCINIUM CORYMBOSUM*) AFFECTED BY IN VITRO DIGESTION**

by

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The biological properties of antioxidants depend on their release from the food matrix during the digestion process. Blueberry contains a wide range of phenolic compounds which are of great significance due to their antioxidant activity. In vitro digestion is a rapid and inexpensive method used to determine the availability of nutrients involved in the absorption studies with humans. Total phenolic content and antioxidant activity of *Vaccinium Corymbosum* blueberry was studied after in vitro digestion. The digested sample showed significantly higher TPC, DPPH and ORAC values compared to undigested sample. Highest antioxidant activity was observed in treated in vitro digested blueberry sample as measured by DPPH and ORAC methods. Results from this study showed that digestion enhances the availability of antioxidants in blueberry, where the digested fruit demonstrate a higher antioxidant content and capacity, with $p < 0.001$ for TPC, $p < 0.05$ for DPPH and $p < 0.05$ for ORAC.

AUTOBIOGRAPHICAL STATEMENT

Sonia Gharbi received her food engineer diploma from National Institute of Agronomy of Tunis, Tunisia in 2009. In 2012, she joined Wayne State University (WSU) and is currently completing her graduate studies toward the accomplishment of master of Nutrition and Food Science degree.