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Antioxidant Activity In Green And Roasted Coffee: A Critical Review

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The pleasant taste and aroma coffee make it one of the most popular and widely consumed beverages worldwide. A number of studies have been performed to elucidate the possible beneficial effects of coffee consumption on human health and have shown that coffee exhibits potent antioxidant activity, which may be attributed mainly to its polyphenolic content. However, there is also evidence to suggest that coffee roasting (the procedure which turns green coffee beans to the dark, roasted ones from which the beverage derives) may alter the polyphenolic profile of the beans (e.g., via the Maillard reaction) and, concomitantly, their antioxidant activity. In the present study, the main findings on difference of antioxidant effect of green coffee and roasted coffee in vitro, cell-free and cell-based assays, animal and human studies is reviewed and summarized.

Keywords: Antioxidant; Green coffee; Roasted coffee; Oxidative stress; Free radicals

INTRODUCTION

Since centuries plants were used as the oldest biofactory in human life as food and medicine. Different part of plants has been used as part of conventional traditional medicines based on their rich content of bioactive metabolites which help in the prevention and in the treatment of different diseases (Gomaa et al. 2019; Taher et al.2019). Free radicals are most commonly defined as “molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals”. (Pala and Gurkan, 2008) They are short-lived, uncharged and very reactive molecules. Examples of free radicals in human body are hydroxyl radical, alkyl-oxy radical and superoxide anion. These radicals attack each and individual cell of our body. Studies have confirmed that excessive accumulation of free radicals cause significant damage to the human body resulting in numerous diseases and premature aging. The damages caused by free radicals are collectively referred to as oxidative stress. Examples of the damages are oxidization of blood vessel walls, protein molecules, DNA, and carbohydrates. The free radicals also interact with membrane lipids that contain unsaturated bondsand thus change the properties of cell membranes. This changeaffects the way the cells interact with their environment and reproduce, hence affect cell

function and result in a variety of diseases such as cardiovascular diseases, cancer and diabetes. Oxidative stress also plays a key role in the pathogenesis of aging. (Kameya, 2017)

Oxidative stress can be reduced by antioxidants. Antioxidants are defined as chemicals that bind with free radicals and nullify their effect from causing damage to biological molecules. The human body produce endogenous antioxidants to combat various free radicals such as Glutathione (GSH), Alpha Lipoic Acid (ALA), Superoxide Dismutase (SOD), Catalase and Coenzyme Q10 (CoQ10). (Aguilar et al. 2016) However, human body still needs antioxidants from external sources, primarily through diet called as exogenous antioxidants or dietary antioxidants contained in vegetables, fruits, berries, vegetable oils, honey, tea, coffee, cocoa, juices, wine, sprouted grains, and other foods (Cappelletti et al. 2015; Mustafa et al. 2016; Selvamani et al.2018; Haque et al.2019).

Coffee is one of the major sources of antioxidants in many peoples' daily diet. Liang and Kitts (2014) described in vitro, cell-free and cell-based assays that both characterize and compare the antioxidant capacity and mechanism of action of coffee and its bioactive constituents. They also reviewed evidence of cellular antioxidant activity induced by coffee components, which are relevant to antioxidant function in both animal and human studies.

Research on the potential protective effects of coffee and its bioactives (caffeine, chlorogenic acids and diterpenes) against oxidative stress and related chronic disease risk has been increasing in the last years. The effect of coffee consumption on protection against lipid, protein and DNA damage, as well as on the modulation of antioxidant capacity and antioxidant enzymes in human studies has been reviewed and the results suggest that coffee consumption can increase glutathione levels and improve protection against DNA damage, especially following regular/repeated intake. (Martini et al. 2016) Reviews on the health effects of antioxidants in coffee have been published in areas of mental performance, sports performance, antimicrobial, type 2 diabetes, liver disease, neurodegenerative disorders, cancer, antiaging and cardiovascular disease (CVD) (Carlström and Larsson, 2018).

The antioxidant effect of coffee is exerted by its rich content of bioactive compounds especially polyphenols, such as phenolic acids, mostly chlorogenic (in green beans) and caffeic (occurring after roasting). Other phenolic acids in coffee beans are ferulic and p-coumaric. Although we are aware that coffee beans undergo roasting prior to consumption, recent research compares antioxidant activities in green coffee and roasted coffee. This article summarizes the difference in antioxidant activity between green coffee beans and roasted coffee beans (Król et al. 2020).

ANTIOXIDANTS IN COFFEE

Coffee, both green and roasted, contains many bioactive compounds that exert powerful antioxidant activity. The antioxidant activity of coffee varies based on many different factors such as coffee bean type and variety, region of cultivation, and roasting process. Recent research shows the significant differences in the bioactive compounds between green and roasted coffee (Król et al. 2020).

The main bioactive compounds that have antioxidant activity in coffee are under phenolic group. However, it was reported that the main polyphenol in coffee is chlorogenic acid. Coffee also contains a significant amount of nicotinic acid, trigonelline, tocopherols, cafestol, kahweol, and heterocyclic compounds. (Król et al. 2020).

Chlorogenic Acid

Chlorogenic acid (CGA) (Figure 1), is an ester that is formed from the reaction of caffeic acid with L-quinic acid; hence the name 5-caffeoylquinic acid (5-CQA). It is a polyphenolic compound that is abundant in many plants. Tobacco leaves, mulberry tree, and coffee beans contain CGA and is found to be responsible for the astringent taste of coffee brews. The underlying mechanism for specific health benefits attributed to CGAs involves mitigating oxidative stress, and hence the related adverse effects associated with an unbalanced intracellular redox state. CGAs donate hydrogen atoms to reduce free radicals and to inhibit oxidation reactions which give it antioxidant effect. After donating hydrogen atoms, CGAs are oxidized to respective phenoxyl radicals and these phenoxyl radicals are quickly stabilized by resonance stabilization.

Some studies have indicated that CGAs may lower blood sugar levels (Eamon et al. 2011), Lowering body fat content by reducing levels of triglycerides, LDL-cholesterol, and VLDL (Shengxi et al. 2013), preventing the absorption and production (by inhibiting fatty acid synthase) of fats, while increasing their breakdown (by stimulating beta-oxidation) (Cho et al. 2010) and inhibiting acetylcholinesterase,resulting in improved cognition and memory(Kwon et al.

2010). In addition, CGAs also exhibit anti-inflammatory activities by modulating a number of important metabolic pathways (Liang and Kitts, 2016).

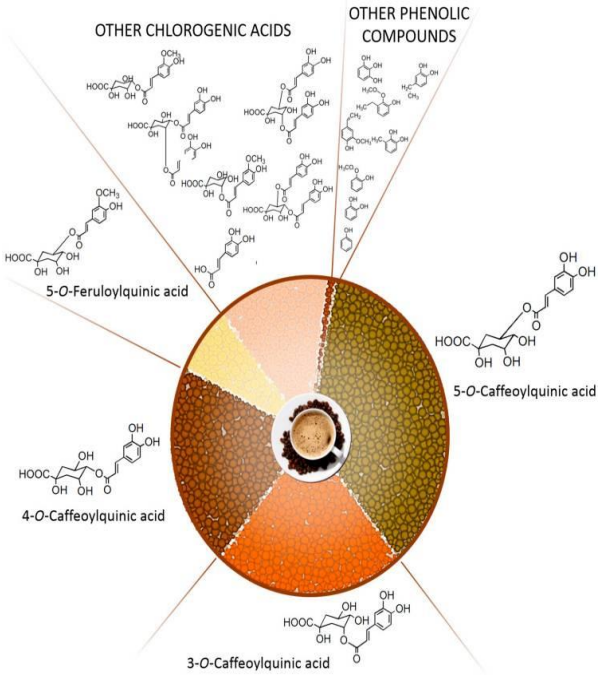

Figure 1: Coffee phenolic compounds (Tresserra-Rimbau et al., 2014)

The most abundant CGA in green coffee beans is 5-CQA, which accounts for 76%-84% of the total CGAs, or approximately 10 g/100 g coffee beans. The total CGAs content and profile is affected significantly by roasting conditions. The more the coffee beans are roasted, the lower the content of total CGAs. Around 45%-54% of CGAs were lost in the light roasted (230 ºC, 12 min) beans compared to the green beans, whereas more than 99% of CGAs were lost in higher roasts (such as city roast, 250 ºC for 17 min; French roast, 250 ºC for 21 min) (Liang and Kitts, 2016).

Diterpenes

Diterpenes that are most commonly found in coffee are cafestol and kahweol. Both cafestol and kahweol from coffee exhibit up to 70% free radical scavenging activity at 400 ppm concentration. Roasted beans exhibit free radical scavenging activity 5% less than green beans. Generally, lipid peroxidation causes cell damage and cell death and also produces undesirable color, odour and taste to foods. Cafestol and kahweol exhibit 70% lipid peroxidation inhibition at 400 ppm concentration coffee brew sample. Studies show protective effect of coffee diterpenes on ter-butylhydroperoxide induced oxidative hepatotoxicity and protection against carcinogens as demonstrated in animal models, liver damage and inhibition of metastasis (Sridevi and Giridhar, 2015).

Melanoidins

During coffee roasting process, majority of phenolic compounds are destroyed or they may react with free radicals from the Maillard reaction and be incorporated in browning products. Thus, the significantly higher oxygen scavenging properties of roasted coffee brews can be attributed to the Maillard reaction products (MRPs), especially melanoidins formed during the thermal treatment, although the remaining caffeic and chlorogenic acids and/or their degradation products may exhibit antioxidant capacity as well (Komes and Bušić, 2014).

Melanoidins are one of the major components of coffee brews, accounting for up to 25% of dry matter. They are responsible for the strong antioxidant properties and metal-chelating ability showed by coffee brews. This effect is due to their ability to break the radical chain by donation of hydrogen, effectivity as metal-chelating agents, capacity to reduce hydroperoxide to nonradical products, or to scavenge hydroxyl radicals. High-molecular-weight polymerized melanoidins in coffee brews contribute to the antioxidant capacity less than the presence of low-molecular-weight MRPs. All roasted coffee brews showed higher oxygen scavenging capacity than the green coffee brew due to presence of melanoidins which is not found in green coffee. (Delgado-Andrade and Morales, 2005; Komes and Bušić, 2014).

Caffeine

Caffeine is an alkaloid present in varying amounts in brewed coffees, mostly known for its specific effects on increased mental alertness, faster information processing, wakefulness, restlessness, reduction of fatigue, and delay in the need for sleep. The caffeine content in brewed coffee depends on the type of bean, brewing strength, and roasting process. Regarding the green beans, the highest and the lowest caffeine contents are found in the samples of highest and lowest quality, respectively. The same tendency is maintained after roasting. Caffeine content of green coffee beans is within the range of 1-4% (on dry basis) while the roasting causes a reduction in caffeine content of approximately 30%. Caffeine presents a distinct behavior for 200°C and 300°C roasting conditions, showing a sharper decrease for roasting at 300°C. Since the

solubility of this compound in water increases with temperature, the caffeine loss may be attributed to a drag by water vapor released during roasting (Mazzafera and Silvarolla, 2010).

Caffeine and its catabolic products theobromine and xanthine exhibit both antioxidant and pro-oxidant properties. Therefore, caffeine and its metabolites may also contribute to the overall antioxidant and chemo preventive properties of caffeine-bearing beverages (Vignoli et al. 2011). Caffeine's main effect is on the brain. It stimulates the brain by locking the effects of the neurotransmitter adenosine (Ferre, 2008). This causes a relative increase in other signaling molecules, such as dopamine and norepinephrine which is thought to benefit mood and brain function (Nehig et al.1992).

Tocopherols

Tocopherols are a group of four lipid-soluble amphipathic molecules (α-, β-, γ-, δ-) that are exclusively synthesized by photosynthetic organisms. Collectively, they are an essential component of vitamin E which is known to be the most effective natural lipid-soluble antioxidant, protecting cell membranes from peroxyl radicals and mutagenic nitrogen oxide species (Alves et al. 2010).

Among tocopherols, two main tocopherols (α- and β-) were identified in both green and roasted of Arabica and Robusta coffee beans. Vitamin E mean content in coffee brew was found to be 7±3 µg/100 ml and α-tocopherol content in roasted coffees ranged between 7.55 µg/g and 33.54 µg/g, whereas in green coffees it was reported to be ranged from 2.02 µg/g to 16.76 µg/g. In the case of β- and γ-tocopherols, remarkable differences between green and roasted samples were observed, with their contents being higher in roasted coffees. Thus, the mean values of β-tocopherol were evaluated to be 47.12 µg/g and 106.60 µg/g for green and roasted coffees, respectively. In the case of γ-tocopherol, its content varied between 2.63 µg/g in green samples and 70.99 µg/g in roasted coffee beans. The increase in tocopherol content during the roasting process was explained as the result of liberation of the combined tocopherols during the roasting process (Alves et al. 2010).

Tocopherol is a potent antioxidant, which can help prevent and fight the damage caused by free radicals. In fact, studies have demonstrated that tocopherol not only helps to reduce internal inflammation but also inflammation of the skin. (Nachbar and Korting, 1995). Tocopherol may boost the energy by promoting better blood circulation and strengthening the capillary walls, which nourishes the cells (Takanami et al. 2000).

EVALUATION OF ANTIOXIDANT ACTIVITY IN COFFEE

DPPH assay

2,2-diphenyl-1-picrylhydrazyl (DPPH), is a stable free radical with an unpaired electron that is delocalized over the entire molecule and thus, employed in the DPPH assay. DPPH possesses a purple color, with a maximum absorption at 519 nm in ethanol hence, scavenging the DPPH radical by coffee antioxidants will result in a decrease in absorption readings over time; the extent of decrease in DPPH absorption being proportional to the concentration of radicals that are being scavenged, according to the principle of Blois. Measurements are made using a UV-visible spectrophotometer at room temperature, and the scavenging capacity is represented as the percentage of DPPH radical inhibition. The DPPH assay is based on both electron transfer (SET) and hydrogen atom transfer (HAT) reactions. An advantage of the DPPH assay is that it is an easy, economic and rapid method to evaluate the radical scavenging activity of non-enzymatic antioxidants (Kedareand Singh, 2011).

Since DPPH is a stable radical, this assay considers not only the concentration of the tested sample, but also the reaction time and the temperature; both of which when controlled carefully enable this assay to be highly reproducible. There are, however, limitations to this assay when used to measure the antioxidant activity of brewed coffee are related to the color of the coffee, thus potentially interfering with the DPPH absorption. Furthermore, DPPH is a lipophilic radical with limited accessibility to the hydrophilic components present in brewed coffee, thereby requiring alcohol in the reaction mixture to ensure maximum solubility. The presence of ethanol adds to a background antioxidant activity, which needs to be considered when standards are constructed for quantification purposes. Proteins, if present in the brewed coffee will also interfere with the assay once precipitated by the presence of ethanol in the reaction system. The biggest limitation of the DPPH assay is that it is not related to specific free radicals that have physiological relevance (Kedare and Singh, 2011).

ABTS assay

The ABTS assay utilizes a free radical, mono-cation of 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), which is generated when ABTS substrate is oxidized with potassium persulfate. ABTS•+ solution has a blue/green color with maximum absorption spectra at 734 nm in water. The more hydrophilic free radical, namely the pre-generated ABTS•+ is decolorized when reduced in the presence of the test sample. This event indicates the extent of relative radical scavenging activity expressed as a percent inhibition. Alternatively, this chemical response can also be compared to a water-soluble vitamin E analogue, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) when tested under the identical conditions. The results are then expressed as TEAC (Trolox equivalent antioxidant capacity). The results of the ABTS assay should be comparable to results found in the DPPH assay and may be viewed as confirmation of the DDPH assay, albeit that absolute values from the ABTS assay are generally higher. Both radicals show the same stoichiometry with water-soluble vitamin E analogue, Trolox (such as two moles of ABTS•+ or two moles of DPPH radicals are scavenged by one mole of Trolox) (Shalaby and Shanab, 2013).

However, ABTS has an advantage to the DPPH assay since ABTS can be used at different pH values and therefore takes into consideration the effect of pH on the antioxidant activity of the tested sample. The ABTS assay is also applicable to testing antioxidant compounds that have a lipophilic or hydrophilic character, which is particularly important when testing different coffee varieties or isolated fractions collected from brewed coffee. The limitation of the ABTS assay, however, involves both possible color interference from the coffee browning pigments present in the brew and, as is the case with the DPPH assay, produces results that have little physiological relevance to naturally produced unstable radicals. In general, highly pigmented and hydrophilic antioxidants are better assessed for antioxidant activity using the ABTS assay compared to the DPPH assay. Since roasted coffee is rich in pigments and possesses hydrophilic components in a complex matrix derived from both brewed coffee and extracts recovered from spent coffee, the ABTS assay enables more complete information on the relative antioxidant capacity of different coffees or related bioactive constituents (Shalaby and Shanab, 2013).

FRAP and TRAP assays

The ferric ion reducing antioxidant power (FRAP) assay is a non-specific, redox-linked, colorimetric assay that is related to the molar concentration of the antioxidant present. This assay is performed based on the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions at low pH buffer which resulted in the formation of coloured ferrous-tripyridyltriazine complex and thus quantitated at absorption maximum of 593 nm. The increase in absorbance at 593 nm is proportional to the total ferric reducing power of the tested sample. Results are presented as mg of Fe^{2+}/g of dried sample and represent the mass of Fe^{3+}, which can be reduced by 1 g of dried sample. Although the FRAP assay was originally developed to measure the antioxidant power of plasma, this highly reproducible and inexpensive assay has also been used to assess the total antioxidant capacity of different food systems including coffee (Valadao et al. 2014).

When interpreting the results of this test, it is important to understand that this assay measures the capacity of a sample to participate in one-electron redox reactions hence, other antioxidant compounds present in the coffee, which may possess different modes of action (e.g., radical scavengers), will not be included in the reaction. Although the assay is compatible with coffee beverages, which contain mostly water-soluble components that react in the aqueous solution, the reducing capacity is determined in the absence of molecules that may have protective capacities to detoxify reactive oxygen species. FRAP also does not measure potentially important coffee antioxidant components that contain thiol groups, due to a redox potential threshold that is below FRAP detection. Moreover, non-activity interaction has been reported between different antioxidants when FRAP is used. FRAP, and other SET-based assays, have in common a limitation that they only reflect reducing capacity and, therefore, do not identify potential antioxidants that work through a HAT mechanism. Since most phenolic compounds, such as chlorogenic acid in coffee, exhibit antioxidant activity by hydrogen donation, SET-based assays, such as DPPH and FRAP, are limited in quantitatively describing the antioxidant activity of coffee beverages (Valadao et al. 2014).

Unlike the FRAP assay, the TRAP (total peroxyl radical-trapping antioxidant parameter) is based on the application of the thermal decomposition of water-soluble, azo-compound 2,2'-azobis-(2-amidinopropane hydrochloride) to

peroxyl radicals. Spectra are recorded over a 10–15 min period at frequent (90 s) intervals, and the absorbance at 734 nm is measured as a function of time. In all of these assays, the unit of activity can be expressed as a Trolox equivalent antioxidant capacity (TEAC) or defined as the concentration (mM) of Trolox having equivalent antioxidant capacity to a 1.0 mM solution of the substance under study. TEAC values derived from different antioxidant components, such as vitamin C and vitamin E, are considerably different, thus this assay has severe limitations to account for the antioxidant capacity of coffee beverages, since they represent relatively complex mixtures of individual components that share antioxidant activity (Valadao et al. 2014).

ORAC assay

The oxygen radical antioxidant capacity (ORAC) assay is used to evaluate the capacity ofantioxidant compounds that scavenge peroxyl radicals generated by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). This method, originally described by Cao and others, is a popularly used HAT method to evaluate model Maillard reactions and coffee constituents. The assay originally used β-phycoerythrin as an indicator protein and AAPH as a peroxyl radical generator. AAPH constantly generates peroxyl radicals, which, in turn, oxidizes β-phycoerythrin, thus reducing the fluorescence intensity of β-phycoerythrin. The assay therefore measures the extent to which theantioxidant sample protects the β-phycoerythrin from oxidation in the presence of AAPH. The protective effect of the tested sample is measured from the area under the test sample fluorescence decay curve, as compared to that of the blank. β-phycoerythrin has a limitation due to nonspecific binding with polyphenols; hence, Ou and others replaced β-phycoerythrin with fluorescein in a modified ORAC assay that evaluates more accurately the peroxyl radical scavenging capacities of aqueous soluble components present in coffee (Liu and Kitts, 2012).

ORAC assay is not only suitable for measuring the antioxidant capacity of the hydrophilic components in brewed coffee, but can be adjusted todetermine the antioxidant capacity of hydrophobic components by changing the buffer system.This advantage enables quantitation of antioxidant capacity for both hydrophilic and hydrophobiccomponents in coffee. In addition, the assay reflects the anti-peroxyl radical capacity of antioxidants that may have different free radical scavenging rates and reaction kinetics. ORAC is therefore suitable to study the antioxidant capacity of brewed coffee, which is a complex mixture of many component having different reaction kinetics. Extrapolation of ORAC results to whole bodyredox status is limited by the fact that both the oxidation rate and characteristics of fluorescein in the presence of peroxyl radical only mimic the reaction subtracts in vivo (Liu et al. 2012).

Hydroxyl radical scavenging assay

The hydroxyl radical is one of the most reactive free radicals in a biological system. Therefore, it is important to consider the hydroxyl radical scavenging capacity of dietary antioxidants. Hydroxylradicals can be generated by Fenton reaction between ferrous iron and H_2O_2. Hydroxyl radicalsare also generated by the reaction between DMSO and H_2O_2. Different probes, such as deoxyribose, benzoate and salicylate, have employed colorimetric or fluorometric measures to indicate the damage caused by hydroxyl radicals. PM2 bacteriophage DNA has also been used to show the protection of coffee constituents against hydroxyl radical-induced single- and double-strand DNA scission. Hydroxyl radical scavengers present in tested samples protect the probe from being damaged by hydroxyl radicals. The percentage of hydroxyl radical scavenging activity of test sample is determined in comparison with a negative control. In addition to using probes, hydroxyl radical can also be quantified by electron paramagnetic resonance (EPR) with the help of aspin-trap agent. Spin trapping is a technique where a nitrone- or nitroso- compound reacts with a targetfree radical to form a relatively stable adduct, which is measurable with EPR spectroscopy and gives a distinguishable EPR spectrum (Naidu et al. 2008).

The hydroxyl radical scavenging capacity assay has been used to study green coffee. A potential limitation of this assay for measuring the antioxidant activity of coffee is that coffee components can chelate transition metal ions, such as Fe^{2+} and, consequently, interfere with the Fenton reaction, which normally generates the hydroxyl radical. Therefore, it is difficult to characterize the antioxidant activity of coffee components whether they are scavenging the hydroxyl radical directly or acting indirectly by chelating Fe^{2+}. Another limitation of this procedure has to do with the fact that roasted coffee beans and brewed coffee contain H_2O_2, a precursor

component of the Fenton reaction (Naidu et al., 2008).

Superoxide radical scavenging capacity assay

The oxygen-scavenging capacity assay involves using a superoxide radical anion (O_2^-) that is generated through either enzymatic or non-enzymatic O_2^- reaction systems. In the enzymatic system, xanthineoxidase (XOD) utilizes hypoxanthine or xanthine as the substrate and O_2 as a cofactor to produce O_2^- and uric acid. The generation of O_2^- in situ, by electron transfer from NADH to O_2 present insolution, is analogous to NADPH oxidase- and NADH dehydrogenase-catalyzed generation in vivo. For a non-enzymatic system, phenazine methosulfate is used to oxidize nicotinamide adenine dinucleotide to generate O_2^-, nitro blue tetrazolium (NBT) is used as the probe to quantitate the oxygen concentration. Oxygen reduces NBT to a purple formazan. After incubation of tested samples with the phenazine methosulfate-NADH-NBT mixture, the absorbance at 562 nm is measured againsta blank to determine the degree of O_2^- scavenging. EPR spectroscopy is a well-defined alternativemethod to measure superoxide radical concentration. Similar to hydroxyl radical, O_2^- is a short-lived free radical and requires DMPO to form a DMPO-OOH adduct with O_2^-, which can then be detected by EPR spectroscopy (Gunalan et al. 2012).

The limitation of DMPO is that it does not distinguish between superoxide and hydroxyl radicals due to the spontaneous decay of DMOP-OOH adduct into DMPO-OH adduct. As a result, other spin traps, such as BMPO, are used to extend the half time without worrying about fast decomposition from BMOP-OOH to BMOP-OH. The superoxide radical scavenging capacity of selected coffee varieties has been evaluated. Oxygen is poorly reactive and can easily decompose to form more potent and reactive free radical species, such as hydroxyl radical (HO^-) and peroxynitrite ($ONOO^-$). Moreover, constituents that are pigmented or have fluorescence in the coffee sample might further interfere with the colorimetric or fluorometric responses. The EPR spectroscopy equipped with an appropriate spin trap agent will give more reliable results compared to fluorometric probes to measure the level of oxygen after reacting with/ without coffee beverage (Gunalan et al. 2012).

Coffee components with antioxidant capacity (cellular antioxidant activity assays and animal studies)

Although chemical-based antioxidant assays are useful for screening food constituents for antioxidant activity, the results cannot be totally extrapolated to biological systems, because antioxidant capacity is not limited to only free radical scavenging ability and reducing capacity, but also includes the activation of redox transcription factors and up regulation of genes that induce the expression of antioxidant enzymes. Cellular antioxidant activity assays provide biologically relevant methods to measure the activity of antioxidants at the cellular level, because they account for important factors, such as cellular uptake, distribution and metabolism. To show the antioxidant activity of coffee components in cell-based model systems, investigators have used a number of different cell lines, stimulators of oxidative stress and various endpoint measures that point to specific underlying mechanisms of antioxidant activity or oxidative stress. The oxidative stress has been induced by different agents such as chemical (AAPH, hydrogen peroxide and tert-Butyl hydroperoxide (t-BOOH)) and also physical (radiation and hyperoxia) stimulation (Zhang et al. 2013).

In previous study by Budryn et al. (2017), the coffee extracts were evaluated in vitro in terms of scavenging capacity, influence on lipids accumulation in 3T3L1 adipocytes, cytoprotective potential decreasing oxidative stress induced by cellular pro-oxidants in βTC3 cells, and in vivo by selected physiological indices in rats fed diets supplemented with the extracts. Roasted coffee extracts, in particular hydrophilic fraction containing monochlorogenic acids and Maillard reaction products, showed the highest scavenging capacity and contributed to the highest level of antioxidants in the hydrophilic fraction of rats serum and may be potentially used as anticancer nutraceutical with oxidative stress inhibitor activity. Non-fractionated green coffee extract exhibited antidiabetic activities including inhibition of adipogenesis, while in vivo it significantly lowered the levels of triglycerides, glucose and oxidized glutathione (Budryn et al. 2017).

Actual measures of cellular metabolism resulting in changes in oxidative stress include determining the concentrations of reduced glutathione (GSH), the presence of a secondary lipid oxidation product, malondialdehyde (MDA), and activities of antioxidant enzymes glutathione peroxidase (GPx), glutathione reductase (GR) and

superoxide dismutase (SOD). GSH is an important intracellular antioxidant that prevents damage from cellular ROS, as well as being a substrate for glutathione enzymes. MDA, a product of secondary lipid peroxidation, is a useful marker for indicating the extent of lipid oxidation that defines potential subsequent reactions with macromolecules and secondary oxidation products (Zhang et al. 2013).

Antioxidant activity of coffee in human studies

The antioxidant potential of coffee derived from chemical-based antioxidant activity assays, cellular-based antioxidant activity assays and animal models have stimulated parallel investigation that has focused on determining the influence of coffee consumption on oxidative status in healthy humans and humans with diseases associated with oxidative stress. Antioxidant activity measured in plasma of subjects both before and 1 and 2 h after a 200-mL drink of brewed coffee exhibited significantly higher antioxidant capacity than controls (Natella, 2002). Similarly, 36 healthy human subjects that consumed instant coffee (800 mL/day for five days), co-extracted from green and roasted beans, respectively, also showed a significant decline in the level of lipid peroxidation (e.g., 8-isoprostaglandin F2α) in urine, which corresponded to an increase in the level of serum antioxidant enzymes (glutathione peroxidase and glutathione-S-transferase) (Hoelzl et al. 2010).

However, when the antioxidant activity against oxidative stress between green coffee (GC), rich in chlorogenic acid, and black coffee (BC) was compared, there was significant difference found as shown in Table 3. The research was investigated the effects of GC and BC on cardiovascular markers. The randomized pilot crossover study was performed on healthy subjects who consumed both coffees for 2 weeks. Anthropometry, blood pressure, and arterial elasticity were measured after each intervention and urine samples were collected to monitor antioxidant capacity. Free cortisol and cortisone levels were obtained from urine and analyzed by specific ELISA methods. Systolic blood pressure ($P = 0.018$) and arterial elasticity ($P = 0.001$) were significantly reduced after GC. BMI ($P = 0.04$ for BC; $P = 0.01$ for GC) and abdominal fat ($P = 0.01$ for BC; $P = 0.009$ for GC) were also significantly reduced with no changes in energy intake. Urinary free cortisol was significantly reduced from 125.6 ± 85.9 nmol/day to 76.0 ± 54.9 nmol/day following GC and increased to 132.1 ± 89.1 nmol/day after BC. Urinary free cortisone increased by 18% following BC and 9% following GC (non significant). Cortisol/cortisone ratio (indicating 11β-HSD1 activity) was reduced after GC (from 3.5 ± 1.9 to 1.7 ± 1.04, $P = 0.002$). This suggests that GC can play a role in reducing cardiovascular risk factors (Revuelta-Iniesta & Al-Dujaili, 2014).

COMPARISON OF ANTIOXIDANT ACTIVITY IN GREEN COFFEE AND ROASTED COFFEE

Research on the antioxidant activity in green coffee and roasted coffee has been increasing in the last years. The present review summarizes the main findings on difference of antioxidant effect of green coffee and roasted coffee in vitro, cell-free and cell-based assays, animal and human studies. In this research, GC is defined as the coffee beans that have not yet been roasted. This research includes studies involving all types of green coffee and from all regions of cultivation. RC includes all types of coffee from all regions of cultivation with all degrees of roasting temperature and time.

Six studies were identified which compare the antioxidant activity of GC with RC using cell free assays. The results of the studies are summarized in Table 2. Previous studies have found that GC has the highest content of CGA (Priftis et al. 2015; Budryn et al. 2017 and Song et al. 2018). For TPC, (Priftis et al. 2015) and (Acidri et al. 2020) reported that RC has higher content. Contradictingly, (Odžaković et al. 2016) reported that GC has higher TPC. (Herawati et al. 2018) and (Song et al.2018) found that lightly roasted coffee at 185–190 ºC for 12 minutes and 220 ºC for 11 minutes respectively, have the highest TPC.

For antioxidant activity using DPPH, (Budryn et al. 2017) and (Acidri et al. 2020) showed higher antioxidant activity in GC contradicting with the findings by (Priftis et al. 2015), (Song et al. 2018) and (Odžaković et al. 2016). For the test using ABTS, (Song et al. 2018) and (Odžaković et al. 2016) showed higher antioxidant activity in GC. But (Priftis et al. 2015) found the opposite. However, All the studies done on antioxidant activity of GC and RC using OH^-, O^{2-} and FRAP were in mutual agreement that RC showed higher antioxidant activity compared to GC.

As for in vitro cell based assay, (Budryn et al. 2017) and (Daglia et al. 2000) reported that RC has higher oxidative stress scavenging activity in βTC3 cells and rat liver cells respectively as shown in Table 2. Format references as per journal style (Daglia et al. 2000)

Table 1. Antioxidant activities of green coffee (GC) and roasted coffee (RC) tested using different assays

Type of Coffee	TPC	CGA	Result (antioxidant assay)					Conclusion	References
			DPPH	ABTS	TEAC				
					DPPH	ABTS	FRAP		
GC	Mean : 3.8%	2028.4 ppm	as control	as control	NA	NA	NA	GC exhibit high antioxidant activity compared to lightly roasted coffee. Dark roasted coffee has the highest antioxidant activity. Green coffee has the highest CGA content.	Priftis et al., 2015
RB 1 (12 min 30 sec; 210°C)	↑	NA	↑24.1%,	↑30.4%,	NA	NA	NA		
RB4 (12 min; 211°C)	↓	NA	↑15.2%	↑25.2%	NA	NA	NA		
RB7 (12 min; 208°C)	↑	NA	↓26.0%	↓16.4%	NA	NA	NA		
215˚C, 7 min 15 sec (R1),	NA	NA	↑32.8%	↑33.4%	NA	NA	NA		
215˚C,6 min 5 sec (R2),	NA	NA	↑46.2%	↑42.4%	NA	NA	NA		
215˚C,5 min 32 sec (R3)	NA	NA	↑87.9%	↑102.0%	NA	NA	NA		
215˚C,3 min 52 sec (R4)	NA	38.8 ppm	↑99.6%	↑135.0%,	NA	NA	NA		
GC	NA	54.35%	57.33%	NA	NA	NA	NA	GC has higher antioxidant activity	Budryn et al., 2017
RC (230°C;12 min)	NA	13.06%	41.60%	NA	NA	NA	NA		
GC	29.3 mg g^{-1} GAE	NA	146.8 µg/mL)	NA	199.7 µmol Trolox g^{-1})	220.7 µmol Trolox g^{-1})	974.2 (µmol Trolox g^{-1})	RB contain the highest content of the TPC, hence, corresponding higher antioxidant activity	Acidri et al., 2020
RC	35.4 mg g^{-1} GAE	NA	87.0 µg/mL)	NA	337.0 µmol Trolox g^{-1})	325.5 µmol Trolox g^{-1})	1104.4 (µmol Trolox g^{-1})		
GC	14.31g GAE/100g)	NA	3.07 mg/mL	NA	NA	NA	25.13(g TEAC/100 g)	The antioxidant activity is highest in the dark r oasted coffee.	Herawati et al., 2018
early yellow (EY), 185–190˚C	14.39 g GAE/100g)	NA	2.85 mg/mL	NA	NA	NA	26.03 (g TEAC/100 g)		
brown (BR) 190–195˚C	13.38 g GAE/100g)	NA	2.97 mg/mL	NA	NA	NA	30.01 (g TEAC/100 g)		
1st crack done (CD) 195–200˚C	12.84 g GAE/100g)	NA	3.80 mg/mL	NA	NA	NA	29.59 (g TEAC/100 g)		
very light (CR), 200–205˚C	10.30 g GAE/100g)	NA	4.14 mg/mL	NA	NA	NA	24.74 (g TEAC/100 g)		
light (LG) 205–210˚C	9.65 g GAE/100g)	NA	4.11 mg/mL	NA	NA	NA	29.14 (g TEAC/100 g)		
medium (MED), 210–215˚C	9.35 g GAE/100g)	NA	4.46 mg/mL	NA	NA	NA	28.38 (g TEAC/100 g)		
dark (DR) 215–220°C,	9.59 g GAE/100g)	NA	5.12 mg/mL	NA	NA	NA	27.21 (g TEAC/100 g)		
GC	65.92 (mg GAE/g)	15.72 g/100g	1.23 mg/mL	0.87 mg/mL	NA	NA	NA	CGA content is highest in GC . However, the antioxidant activity is highest in MD roasted coffee.	Song et al., 2018
ML-Medium Light; (220℃. 11 min)	81.08 (mg GAE/g)	10.19 g/100g	1.11 mg/mL	0.74 mg/mL	NA	NA	NA		
ME-Medium; (220℃. 12 min)	65.66 (mg GAE/g)	4.37 g/100g	1.23 mg/mL	0.80 mg/mL	NA	NA	NA		
MD-Medium Dark; (220℃. 13 min)	57.59 (mg GAE/g)	1.71 g/100g	1.32 mg/mL	0.82 mg/mL	NA	NA	NA		

GC	31.65 (mg GAE/g)	NA	NA	NA	179.23(µmol TE/g)	160.67 (µmol TE/g)	NA	The TPC is highest in GC but the Antioxidant activity is highest in RC 1	Odžaković et al., 2016
RC 1 (167°C, 25 min)	29.81 (mg GAE/g)	NA	NA	NA	203.38 (µmol TE/g)	211.03 (µmol TE/g)	NA		
RC 2 (175°C, 25 min)	23.66 (mg GAE/g)	NA	NA	NA	176.44 (µmol TE/g)	179.28 (µmol TE/g)	NA		

Table 2. Antioxidant activity of green coffee (GC) and roasted coffee (RC) in vitro (cell based) and animal studies

Cell line / animal	Oxidative stress stimulator	Description of oxidative stress in sample compared to the negative control			Conclusion	References
		TEST	GC	RC		
βTC3 cells	t-BOOH	ROS	↓	↓	Higher antioxidant activity in RC	Budryn et al., 2017
Rat liver cells	Lipid peroxidation	protective activity	↑	↑	Higher antioxidant activity in RC	Daglia et al., 2000
ANIMAL						
Rats	High fat diet	ACW antioxidant capacity of water-soluble substances	↓	↑	Higher antioxidant activity in RC	Priftis et al., 2015
		ACL antioxidant capacity of lipid-soluble substances	↑	↑	Higher antioxidant activity in RC	
		GSH reduced glutathione	↑	↑	Higher antioxidant activity in RC	

Table 3. Antioxidant activities of green coffee (GC) and roasted coffee (RC) in human studies

Subject	Study design	Type of coffee and composition	Dosage	Markers	References
20 subjects (7 males, 13 females), mean BMI 24.23 ± 4.6 kg/m2, age n.d.	Randomized, cross-over intervention	Coffee 1: BC (black coffee): Sainsbury's Original Blend Cafetière Coffee Composition: polyphenols ranging from 1451 mg GAE/100 mL (Filter method) to 2475 mg GAE/100 mL (French Cafetiere)	40 g/day of GC for 2 weeks	= Antioxidant capacity (FRAP)	Revuelta-Iniesta and Al-Dujaili, 2014
		Coffee 2: GC (green coffee): Ethiopian Harrar 4 (100% Arabica) Composition: polyphenols ranging from 972 mg GAE/100 mL (French Cafetiere) to 2052 mg GAE/100 mL (Italian Cafetiere)	40 g/day of BC for 2 weeks		

The only animal study which compares free radical scavenging activity by GC and RC also reported that RC has higher antioxidant activity compared to GC.

GCis rich in high-antioxidant activity compounds like polyphenols, chiefychlorogenic acid. Besides scavenging oxidative stress, Chlorogenic acid also exhibits antiviral and hypoglycaemic properties. Coffee's antioxidant potential comes not only from its natural compounds but also from those that arise during processing and its degradation products (ie. ferulic, coumaric and caffeic acids), together with melanoidin compounds derived from the Maillard reaction through roasting process. This review indicates that the antioxidant activity of GC is lower than in RC. Such effects are due to phenolic compounds being released during roasting, as well as to the action of the non-phenolic fraction.

Antioxidant properties are maintained or even elevated, despite significant decreases in natural antioxidant concentrations arising from thermal processes. Roasting evokes several changes in the constituents of coffee beans through modification or degradation. During roasting, high temperatures result in polyphenol degradation. Chlorogenic, malic and citric acid levels become decreased, whilst quinic acid increases due to chlorogenic acid degradation. Thermal degradation of chlorogenic acid gives rise to phenolic compounds, such as chlorogenic acid lactones, which increase the bitter taste of coffee brews. During roasting, Maillard's reactions generate, a variety of compounds such as melanoidins that create the aroma of coffee, increase antioxidant activity and also colour. Hence, this explains why RC generally has higher antioxidant activity compared to GC. However, the content of polyphenols in GC and RC are different and exert different health benefits (Komes and Bušić, 2014).

CONCLUSION

In conclusion, coffee extracts from green or roasted beans exhibited potent free radical scavenging activity. The differences in the levels of antioxidant activity between green and roasted bean extracts extracted from the same variety were also noted. In some coffee varieties, bean roasting reduced antioxidant activity, whereas in others the opposite was noted. It appears that the final effect depends on the chemical composition of the beans of each coffee variety, but this hypothesis requires further investigation. In addition, roasting time was shown to affect the antioxidant activity of roasted coffee beans. This observation suggests that the roasting time should be optimized in order to maintain the levels of antioxidant activity as high as possible. Understanding the mechanisms through which coffee acts as an antioxidant will lead to improvements in the extraction and roasting processes and the ability to fully exploit its properties..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MY, ZB, HAE, RM involved in data collection and writing the manuscript. ZB, SH, AB, SN designed the work, TH, HAE, DJD reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Acidri R, Sawai Y, Sugimoto Y, Handa T, Sasagawa D, Masunaga T, Yamamoto S, Nishihara E, 2020. Phytochemical profile and antioxidant capacity of coffee plant organs compared to green and roasted coffee beans. Antioxidants 9(2):93.

Aguilar TA, Navarro BC, Pérez JA, 2016. Endogenous antioxidants: A review of their role in oxidative stress. In A Master Regulator of Oxidative Stress - the

Transcription Factor Nrf2; In Tech: Rijeka, Croatia, 3-19.

Alicandro G, Tavani A, La Vecchia C, 2017. Coffee and cancer risk: a summary overview. Eur J Cancer Prev 26(5):424-432.

Alves RC, Soares C, Casal S, Fernandes JD, Beatriz M, Oliveira PP, 2010. Acrylamide in espresso coffee: Influence of species, roast degree and brew length. Food Chem 119:929-934.

Arauz J, Ramos-Tovar E, Muriel P, 2017. Liver pathophysiology therapies and antioxidants. Liver Pathophysiology Chapter 48 - Coffee and the Liver, 675-685.

Bobková A, Hudáček M, Jakabová S, Belej Ľ, Capcarová M, Čurlej J, Bobko M, Árvay J, Jakab I, Čapla J and Demianová A, 2020. The effect of roasting on the total polyphenols and antioxidant activity of coffee. J Environ Sci Heal Part B, 1-6.

Budryn G, Zakłos-Szyda M, Zaczyńska D, Żyżelewicz D, Grzelczyk J, Zduńczyk Z, Juśkiewicz J, 2017. Green and roasted coffee extracts as antioxidants in βTC3 cells with induced oxidative stress and lipid accumulation inhibitors in 3T3L1 cells, and their bioactivity in rats fed high fat diet. Eur Food Res Technol 243:1323-1334.

Cappelletti S, Piacentino D, Sani G, Aromatario M, 2015. Caffeine: cognitive and physical performance enhancer or psychoactive drug? Curr Neuropharmacol. 13(1):71-88.

Carlström M, Larsson SC, 2018. Coffee consumption and reduced risk of developing type 2 diabetes: a systematic review with meta-analysis. Nutr Rev 76(6):395-417.

Cho AS, Jeon SM, Kim MJ, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK, 2010. Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem Toxicol 48(3):937-943.

Daglia M, Papetti A, Gregotti C, Bertè F, Gazzani G, 2000. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. J Agric Food Chem 48(5):1449-1454.

Ferré S, 2008. An update on the mechanisms of the psychostimulant effects of caffeine. J Neurochem. 105(4):1067-1079.

Gomaa SE, Yahayu M, Nurjayadi M, Dailin DJ, El Enshasy H 2019. Antimicrobial Compounds from *Catharanthus roseus*-A Review. Int J Scien & Technol Res 8:113-121.

Gunalan G, Myla N, Balabhaskar R, 2012. In vitro antioxidant analysis of selected coffee bean varieties. J Chem Pharm Res 4:2126-2132.

Haque S, Singh V, Katiyar D, Khan MTA, Tripathi V, El Enshasy H, Pasupuleti M. Mishra BN 2019. Biotransformation of newly synthesized coumarin derivatives by *Candida albicans* as potential antibacterial, antioxidant, and cytotoxic agents. Process Biochem 87:138-144.

Herawati D, Giriwono PE, Dewi FNA, Kashiwagi T, Andarwulan N, 2018. Critical roasting level determines bioactive content and antioxidant activity of Robusta coffee beans. Food Sci Biotechnol 28(1):7-14.

Hoelzl C, Knasmuller S, Wagner KH, Elbling L, Huber W, Kager N, Ferk F, Ehrlich V, Nersesyan A, Neubauer O, Desmarchelier A, Maricel MK, Delatour T, Verguet C, Bezençon C, Besson A, Grathwohl D, Simic T, Kundi M, Schilter B, Cavin C, 2010. Instant coffee with high chlorogenic acid levels protectshumans against oxidative damage of macromolecules. Mol Nutr Food Res 54:1722-1733.

Kameya H, 2017. Evaluation of hydroxyl radical and alkyl-oxy radical scavenging activity of coffee by ESR spin trapping method. J Food Sci Eng 7:305-311.

Kedare SB, Singh RP, 2011. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 48:412-422.

Kobayashi T, Maruyama T, Yoneda T, Miyai H, Azuma T, Tomofuji T, Ekuni D, Morita M, 2020. Effects of coffee intake on oxidative stress during aging-related alterations in periodontal tissue. In vivo 34(2):615-622.

Komes D, Bušić A, 2014. Processing and impact on antioxidants in beverages chapter 3-antioxidants in coffee. Processing and Impact on Antioxidants in Beverages. 25-32.

Król K, Gantner M, Tatarak A, Hallmann E, 2020. The content of polyphenols in coffee beans as roasting, origin and storage effect. Eur Food Res Technol 246:33-39.

Kwon SH, Lee HK, Kim JA, Hong SI, Kim HC, Jo TH, Park YI, Lee CK, Kim YB, Lee SY, Jang CG, 2010. Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice. Eur J Pharmacol 649(1-3):210-217.

Liang N, Kitts DD, 2014. Antioxidant property of coffee components: assessment of methods that define mechanisms of action. Molecules 19:19180-19208.

Liu Y, Kitts DD, 2012. Activation of antioxidant

response element (ARE)-dependent genes by roasted coffee extracts. Food Funct 3:950-954.

Martini D, Del Bo' C, Tassotti M, Riso P, Del Rio D, Brighenti F, Porrini M, 2016. Coffee Consumption and oxidative stress: a review of human intervention studies. Molecules 21(8): 979.

Mazzafera P, Silvarolla MB, 2010. Caffeine content variation in single green Arabica coffee seeds. Seed Sci Res 20:163-167.

Meng S, Cao J, Feng Q, Peng J, Hu Y, 2013. roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evid Based Complement Alternat Med 2013:801457.

Mustafa SM, Chua LS, El Enshasy HA, Abd Majid FA, Abd Malek R, 2016. A critical review on fruit juices probiotication: Pomegranate. Curr Nut Food Sci 12:4-11.

Nachbar F, Korting HC. 1995. The role of vitamin E in normal and damaged skin. J Mol Med (Berl) 73(1):7-17.

Naidu M, Sulochanamma G, Sampathu SR, Srinivas P, 2008. Studies on extraction and antioxidant potential of green coffee. Food Chem 107:377-384.

Natella F, Nardini M, Giannetti I, Dattilo C, Scaccini C, 2002. Coffee drinking influences plasma antioxidant capacity in humans. J Agric Food Chem 50:6211-6216.

Nehlig A, Daval JL, Debry G, 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev 17(2):139-70.

Odžaković B, Džinić N, Kukrić Z, Grujić S, 2016. Effect of roasting degree on the antioxidant activity of different arabica coffee quality classes. Acta Sci Pol Technol Aliment 15(4):409-417.

Pala FS, Gurkan H, 2008. The role of free radicals in etiopathogenesis of diseases. Adv Mol Biol 1:1-9.

Priftis A, Stagos D, Konstantinopoulos K, Tsitsimpikou C, Spandidos DA, Tsatsakis AM, Tzatzarakis MN, Kouretas D, 2015. Comparison of antioxidant activity between green and roasted coffee beans using molecular methods. Mol Med Reports 12(5):7293-7302.

Rafferty EP, Wylie AR, Elliott CT, Chevallier OP, Grieve DJ, Green BD, 2011. *In vitro* and *in vivo* effects of natural putative secretagogues of glucagon-like peptide-1 (GLP-1). Sci Pharm 79(3):615-621.

Revuelta-Iniesta R., Al-Dujaili EAS, 2014. Consumption of green coffee reduces blood pressure and body composition by influencing 11β-HSD1 enzyme activity in healthy individuals: a pilot crossover study using green and black coffee. BioMed Research International, Volume 2014, 9 pages.

Rodriguez-Artalejo F, Lopez-Garcia E, 2018. Coffee consumption and cardiovascular disease: a condensed review of epidemiological evidence and mechanisms. J Agric Fd Chem 66(21):5257-5263.

Santini A, Ferracane R, Mikusová P, Eged S, Srobárová A, Meca G, Mañes J, Ritieni A, 2011. Influence of different coffee drink preparations on ochratoxin A content and evaluation of the antioxidant activity and caffeine variations. Food Control 22:1240-1245.

Selvamani S, El-Enshasy HA, Dailin DJ, Abd Malek R, Hanapi SZ, Ambehabati KK, Sukmawati D, Leng OM, Moloi N (2018). Antioxidant compounds of the edible mushroom *Pleurotus ostreatus*. Int J Biotechnol Wellness Ind 7, 1-14.

Shalaby EA, Shanab SMM, 2013. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of spirulinaplatensis. Indian J Geo-Mar Sci 42:556–564.

Song JL, Asare TS, Kang MY, Lee SC, 2018. Changes in bioactive compounds and antioxidant capacity of coffee under different roasting conditions. Korean J Plant Res 704-713.

Sridevi V, Giridhar P, 2015. Antioxidant potential of free diterpenescafestol and kahweol rich extractives of coffee beans. CJBAS 03(03):78-84.

Taher ZM, Agouillal F, Lim JR, Marof AQ, Dailin DJ, Nurjayadi M, Razif ENM, Gomaa SE, El Ensahsy H, 2019. Anticancer molecules from *Catharanthus roseus.* Indones J Pharm 30:147-156.

Takanami Y, Iwane H, Kawai Y, Shimomitsu T, 2000. Vitamin E supplementation and endurance exercise: are there benefits?. Sports Med 29(2):73-83.

Tresserra-Rimbau A, Medina-Remón, A, Estruch R, Lamuela-Raventós RM, 2014. Coffee polyphenols and high cardiovascular risk parameters. In coffee and health and disease prevention Preedy, V.R., Ed.; Academic

Press: San Diego, CA, USA, 387–394.
Valadao VSJ, Queiroz YS, Gotlieb SLD, da Silva Torres EAF, 2014. Stability of phenolic compounds and antioxidant capacity of regular and decaffeinated coffees. Braz Arch Biol Technol 57:110-118.
Yamagata K, 2018. Do coffee polyphenols have a preventive action on metabolic syndrome associated endothelial dysfunctions? An Assessment of the Current Evidence. Antioxidants 7(2):26.
Zhang D, Xie L, Wei Y, Liu Y, Jia G, Zhou F, Ji B, 2013. Development of a cell-based antioxidant activity assay using dietary fatty acid as oxidative stressor. Food Chem 141:347-356.