International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 08 Issue: 04 | Apr 2021 www.irjet.net p-ISSN: 2395-0072

A Review on The Antioxidant Assays that can be Used to Determine Antioxidant Activity in Coffee.

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Abstract - *Caffeine is commonly found in coffee. Coffee is a chemically complex compound that includes substantial quantities of chlorogenic acid and caffeine. This review summarizes published research on quantifying antioxidant activity in coffee samples in vitro employing multiple methods such as FRAP, ORAC, ABTS, Hydroxyl Radical Scavenging assays. Caffeine is commonly found in coffee. Coffee is a chemically complex compound that includes substantial quantities of chlorogenic acid and caffeine.*

Key Words: Antioxidant, Coffee, Caffeine

1. INTRODUCTION

With this action, the World Health Organization put an end to years of debate over the health effects of coffee. According to new research, coffee reduces asthma and allergy symptoms, prevents tooth decay, increases fat burning, replenishes potassium deficiency, and improves cardiovascular function. Furthermore, both coffee and tea are rich in antioxidants. Coffee is high in dietary antioxidants, and this, along with the fact that it is one of the world's most common drinks, has contributed to the belief that coffee is a significant contributor to dietary antioxidant consumption. Brewed coffee is a dynamic food matrix of various phytochemical components that have antioxidant activity which can scavenge free radicals, donate hydrogen and electrons, have reducing activity, and serve as metal ion pro-oxidant chelators, among other things. Coffee compounds have been shown in more recent research to enable tissue antioxidant gene expression and guard against gastrointestinal oxidative stress. The antioxidant capacity and mode of action of coffee and its bioactive constituents will be characterized and compared using a variety of in vitro, cell-free, and cell-based assays. Furthermore, proof of cellular antioxidant activity and associated unique genomic events caused by coffee components in both animal and human studies, which are important to antioxidant function, would be addressed. Caffeine (1,3,7-trimethylxanthine, C8H10N4O2) is a xanthine alkaloid present in chocolate, tea, cocoa, cola nuts, and other plants. It's one of the most commonly used pharmaceuticals on the planet. Caffeine is consumed by caffeine, tea, chocolate, and cola, and it is often used as a portion. Caffeine is easily ingested into the body after consumption.

1.1 COFFEE

Coffee is a beverage made from processed, roasted coffee beans that is said to be one of the most commonly consumed drinks on the globe. Coffee's appeal is possibly due to its caffeine content, which is praised for its fragrance and taste. Coffee, in reality, is a complex chemical mixture comprising over a thousand different chemicals, including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids, and phenolic compounds, according to studies. The number of human researches on the health benefits of coffee intake are qualitative in nature.

1.2 Antioxidant activity in Coffee

Plant phytochemicals' antioxidant capacity has been suggested as a basis for their beneficial effects against age-related diseases. Coffee is the leading source of antioxidants in the diet, ranking sixth in total antioxidants among 1115 widely consumed foods in the United States. As a result, it's possible that coffee reduces ROS through a direct antioxidant impact. However, it is unclear if antioxidants derived from food offer clear defense against oxidative stress in our bodies. In vivo antioxidant properties of coffee have been shown in a few trials. A substantial drop in lipid peroxidation/isoprostanes was found in two clinical trials investigating the health effects of coffee intake. Furthermore, we previously discovered that coffee consumption is inversely related to death caused by oxidative stress and inflammatory diseases. A broad Finnish intervention trial, on the other hand, showed no signs of lipid peroxidation improvements. Importantly, coffee's antioxidant potential should not necessarily correlate with its biological impact. In laboratory models, the biological effects of dark roasted coffee are stronger than lighter roasted coffee, despite the lower antioxidant content. Some molecules are decreased in concentration while others are produced during the roasting process. About the fact that coffee contains a high volume of antioxidants and some studies indicate decreased biomarkers of oxidative stress, the experimental evidence for coffee's direct antioxidant impact in cancer prevention is poor. Another and more possible pathway through which coffee has biological consequences is by indirect pathways, such as causing endogenous defense through the antioxidant and/or nonantioxidant properties of coffee compounds.

2. IN-VITRO ASSAYS TO DETERMINE COFFEE's ANTIOXIDANT ACTIVITY

2.1 DPPH Assay

The DPPH assay uses 2,2-diphenyl-1-picrylhydrazyl (DPPH), a neutral free radical with an unpaired electron that is delocalized over the whole molecule. Since DPPH has a purple colour and a maximum absorption wavelength of 519 nm in ethanol, scavenging the DPPH radical by coffee antioxidants would result in a decrease in absorption readings over time, with the degree of the decrease equal to the concentration of radicals being scavenged, according to the Blois theory. At room temperature, measurements are taken with a UV-visible spectrophotometer, and the scavenging potential is expressed as a percentage of DPPH radical inhibition. Both electron transfer (SET) and hydrogen atom transfer (HAT) reactions are used in the DPPH assay[18]. The DPPH assay has the benefit of being a simple, inexpensive, and fast way to test the radical scavenging activity of non-enzymatic antioxidants. Since DPPH is a stable radical, this assay takes into account not only the concentration of the measured sample, but also the reaction time and temperature, both of which, when closely regulated, make this assay highly repeatable. However, when used to calculate the antioxidant function of brewed coffee, there are drawbacks to this assay that are related to the color of the coffee, possibly interfering with DPPH absorption. Furthermore, since DPPH is a lipophilic radical with minimal access to the hydrophilic components found in brewed coffee, the reaction mixture must contain alcohol to ensure optimum solubility. The presence of ethanol contributes to the background antioxidant function, which must be taken into consideration when developing criteria for quantification[19]. Proteins in the brewed coffee, if available, would also interact with the assay until ethanol is present in the reaction phase. The DPPH assay's greatest flaw is that it doesn't measure real free radicals with physiological significance.

2.2 ABTS Assay

When the ABTS substrate is oxidized with potassium persulfate, a free radical, mono-cation of 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), is produced. In water, ABTS•+ has a blue/green hue and an overall absorption spectrum of 734 nm. As the more hydrophilic free radical, the pre-generated ABTS•+, is reduced in the presence of the test sample, it decolorizes. This occurrence reflects the percentage inhibition of relative radical scavenging behavior. When measured under the same conditions, this chemical reaction can also be contrasted to Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), a water-soluble vitamin E precursor. After that, the findings are expressed as TEAC (Trolox equivalent antioxidant capacity). [19]The ABTS assay results can be similar to the DPPH assay results and can be used to validate the DDPH assay, though absolute values from the ABTS assay are usually higher. Both radicals have the same stoichiometry with Trolox, a water-soluble vitamin E equivalent (e.g., one mole of Trolox scavenges two moles of ABTS•+ or two moles of DPPH radicals). ABTS, on the other hand, has an advantage over the DPPH assay is that it can be used at various pH values and hence takes into account the impact of pH on the antioxidant behavior of the sample being examined[17]. The ABTS assay can also be used to test lipophilic or hydrophilic antioxidant compounds, which is especially useful when studying various coffee varieties or isolated fractions extracted from brewed coffee. The ABTS assay, on the other hand, is limited by the possibility of color contamination from the coffee browning pigments in the brew, as well as the fact that, like the DPPH assay, it provides findings of no physiological relevance to naturally occurring unstable radicals. In general, the ABTS assay is better for assessing antioxidant activity than the DPPH assay for heavily pigmented and hydrophilic antioxidants. The ABTS assay offers more detailed details on the relative antioxidant ability of various coffees or associated bioactive constituents since roasted coffee is abundant in pigments and includes hydrophilic components in a dynamic matrix obtained from both brewed coffee and extracts retrieved from spent coffee.

2.3 Hydroxyl Radical Scavenging Assay

In a biological environment, the hydroxyl radical is one of the most reactive free radicals. As a consequence, the potential of dietary antioxidants to scavenge hydroxyl radicals is important. The Fenton reaction between ferrous iron and H2O2 will produce hydroxyl radicals. The reaction between DMSO and H2O2 also produces hydroxyl radicals. Different probes also used colorimetric or fluorometric tests to show the harm inflicted by hydroxyl radicals, such as deoxyribose, benzoate, and salicylate. The defense of coffee constituents against hydroxyl radical-induced single- and double-strand DNA scission has also been demonstrated using PM2 bacteriophage DNA[14]. Hydroxyl radical scavengers found in the samples protect the probe from hydroxyl radical damage. In relation to a negative regulation, the percentage of hydroxyl radical scavenging behavior of the test sample is determined. The hydroxyl radical can also be measured using electron paramagnetic resonance (EPR) with the aid of a spin-trap agent, in addition to using probes. Spin trapping is a procedure in which a nitrone- or nitroso-compound reacts with a target free radical to create a sufficiently stable adduct that can be detected using EPR spectroscopy and produces a distinct EPR spectrum[1]. For example, 5,5-dimethyl-1-pyrroline N-oxide (DMPO) is often used to capture short-lived hydroxyl radicals, resulting in reasonably stable DMPO-OH adducts that can be identified and quantified using EPR. However, the stability of the DMPO-OH adduct in the presence of Fe2+ ions must be considered, as Fe2+ ions can rapidly quench the DMPO-OH radical. As a result, choosing the right spin trap is crucial for forming stable spin-adducts in the various environments that characterize reaction systems[13]. Green coffee has been studied using the hydroxyl radical scavenging ability assay. The ability of coffee

components to chelate transition metal ions, such as Fe2+, and thereby interact with the Fenton reaction, which usually produces the hydroxyl radical, is a possible drawback of this assay for calculating the antioxidant activity of coffee. As a consequence, characterizing the antioxidant function of coffee materials, whether they are specifically scavenging the hydroxyl radical or acting indirectly by chelating Fe2+, is challenging. Another drawback to this method is that roasted coffee beans and brewed coffee both contain H2O2, which is a precursor part of the Fenton reaction.

2.4 ORAC Assay

The ORAC assay measures the ability of antioxidant compounds to scavenge peroxyl radicals produced by 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH)[7]. This is a commonly used HAT approach to test model Maillard reactions and coffee constituents, which was first described by Cao and others. -Phycoerythrin was used as an indicator protein and AAPH as a peroxyl radical producer in the initial assay[4]. AAPH continuously creates peroxyl radicals, which oxidise -phycoerythrin and reduce its fluorescence power. In the presence of AAPH, the assay specifies how much the antioxidant sample preserves the -phycoerythrin from oxidation. The region under the test sample fluorescence decay curve relative to the blank is used to determine the defensive effect of the measured sample. Due to the nonspecific binding of -phycoerythrin with polyphenols, Ou and colleagues substituted it with fluorescein [40] in an updated ORAC assay that more reliably assesses the peroxyl radical scavenging capacities of aqueous soluble components present in coffee[14]. Through adjusting the buffer system, the ORAC assay can be modified to assess the antioxidant potential of hydrophobic components in brewed coffee as well. This benefit allows for the calculation of antioxidant potential in both hydrophilic and hydrophobic coffee materials. Furthermore, since the region under the fluorescence decay curve must be measured over a set time span, the assay accurately represents the anti-peroxyl radical potential of antioxidants with varying free radical scavenging speeds and reaction kinetics[13]. The antioxidant potential of brewed coffee, which is a complex mixture of several components with varying reaction kinetics, may therefore be studied using ORAC[17]. The fact that both the oxidation rate and characteristics of fluorescein in the presence of peroxyl radical only resemble the reaction subtracts in vivo limits extrapolation of ORAC effects to whole body redox status.

3. CONCLUSION

In this review, we've summarized the different antioxidant assays and their mode of action. The health hazards and health benefits that are caused by the intake of coffee. Various theories on cancers, as few say that coffee is good for cancer and other say that it causes cancer, whereas it really causes both in different ways. About the fact that the picture is far from simple, suggested mechanisms for coffee's chemo-preventive effects seem to include modulation of DNA repair genes, as well as genes implicated in detoxification, inflammation, apoptosis, angiogenesis, and metastasis.

ACKNOWLEDGEMENT

I would like to thank my guide Dr. Sindhu Anoop who guided and helped us throughout the project, and for her constant support, input, and encouragement. We wish to express our gratitude to the Principal Dr. Sr Arpana and HOD Dr. Preetha Nair who gave us the opportunity to do this review article.

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