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ASSESSMENT OF THE ANTIOXIDANT PROPERTIES OF THE MOST COMMON COFFEE BREWS AVAILABLE IN THE LOCAL MARKETS OF THE WESTERN REGION OF SAUDI ARABIA

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ABSTRACT

The purpose of the current study is to determine the antioxidant activity of the most commonly used coffee brews in the Western region of Saudi Arabia. Further, total phenolic and flavonoid contents were measured and antioxidants properties including ferric reducing antioxidant power and ferrous ion chelating activity, DPPH radical scavenging activity, and scavenging of hydrogen peroxide assays were also determined. Results of study revealed that phenolic contents were 741, 835 and 578 µg/ml of gallic acid/2 g of coffee, and the total flavonoid contents were 711, 802, and 828 µg/ml of catechin/2 g of coffee for Nescafé red mug, Turkish coffee and Espresso, respectively. The inhibition percentage of hydrogen peroxide showed highly significant reduction ($p < 0.001$) in Turkish coffee compared to Nescafé red mug. Further, DPPH activity also showed highly significant reduction ($p < 0.01$) in Espresso and Turkish coffee compared to Nescafé red mug, whereas, Espresso showed a highly significant increase ($p < 0.01$) in reducing power activity. Regarding the metal chelating activity, Espresso showed a significant decrease ($p < 0.05$) in metal chelation activity as compared to Nescafé red mug. The current study demonstrated that Nescafé red mug, Turkish coffee and Espresso, were the most consumed coffee types in the Western region of Saudi Arabia according to the survey. These three types of coffee showed high phenolic and flavonoid contents as well as a high antioxidant activity.

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1 Introduction

Coffee is one of the most popular beverages all over the world, due to its physiological effects as well as its pleasant taste and aroma (Farah, 2012). It is considered as a functional beverage, primarily because of its high content of antioxidant compounds that exert biological beneficial properties (Dórea & da Costa, 2005). Polyphenols are very complex group of molecules present in plants (Scalbert et al., 2002; Yardim, 2012). Several compounds can be identified under the term “polyphenols”; these include mainly phenolic and flavonoids acids (Samanidou, 2014). Antioxidants such as polyphenols are important for the maintenance of health and they also provide protection against many diseases such as coronary heart disease and cancer by preventing oxidative stress (Karakaya et al., 2001). Recently, an international interest has been raised on dietary polyphenols for their considerable role in the prevention of various degenerative diseases. This considerable role is based on some epidemiological and clinical studies and on numerous animal studies (Scalbert et al., 2002).

Drinking coffee on regularly basis may prevent cardiovascular diseases and reduce risk of developing type 2 diabetes, Parkinson's disease, stroke and Alzheimer's disease (Butt & Sultan, 2011). Moreover, coffee has some modest laxative effect also. It can prevent the formation of gallstones, gout and gallbladder diseases by reducing the level of uric acid in blood, which is more significantly observed in men than in women (Dasgupta & Klein, 2014). From this point of view, the aim of this study was to perform a survey to identify the most consumed types of coffee in Jeddah, Western region of Saudi Arabia, and then estimate the antioxidant activities for these most commonly consumed coffee types identified by this survey.

2 Materials and Methods

2.1 Materials

Folin-Ciocalteu's phenol reagent and ferric chloride anhydrous were purchased from (BDH, Poole, UK). 2,2-diphenyl-1-picrylhydrazyl, ethanol, trichloroacetic acid (TCA), ferrous chloride, methanol, ferrozine, gallic acid and sodium nitrite were obtained from (Sigma-Aldrich, Poole, UK). Sodium hydroxide pellets were purchased from (PanReacAppliChem, Barcelona, Spain). Aluminum chloride was purchased from (LOBA, Mumbai, India). Ethylenediaminetetraacetic acid (EDTA) was bought from (Fluka, BUCH, Switzerland). Anhydrous sodium carbonate, sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate were purchased from (CDH, New Delhi, India). Phosphate buffer saline (PBS) and Hydrogen peroxide were obtained from (Oxoid, Hampshire, UK). Vitamin C and potassium ferrocyanide were purchased from (FlukaChemika, Buchs, Switzerland). (+)-catechin was purchased from (Steinheim, Germany).

2.2 Sample preparation

To estimate the type of coffee that is most commonly consumed, a survey was performed on 900 participants. The survey revealed that the most consumed three types of coffee were Nescafé red mug, Turkish coffee and Espresso. The Nescafé red mug coffee was prepared by adding and mixing 2g of Nescafé red mug coffee to 200 ml boiling water. For the Turkish coffee, 3.5g of Turkish coffee was added to 75 ml cold water (Karakaya et al., 2001) and mixed well on a heater until the coffee formed small bubbles. For Espresso, 10 g of coffee was mixed with 180 ml boiling water.

2.3 Determination of total phenolic content

The total phenolic contents in coffee samples were determined by following Folin-Ciocalteu method (Przygodzka et al., 2014) for each coffee type. A standard curve was prepared from a serial dilutions of gallic acid (GA) (0, 50, 100, 150, 200, 250 and 500 µg/ml) and was used to determine the total phenolic contents.

2.4 Determination of total flavonoid content

The total flavonoids content of the coffee samples was determined by aluminum chloride colorimetric assay (Dewanto et al., 2002) for all three coffee types. The absorbance of the final product was measured at 510 nm and the standard curve was prepared from different concentrations of catechin (0, 50, 100, 150, 200, 250, 300 and 500 µg/ml).

2.5 Ferric reducing antioxidant power and ferrous ion chelating activity assay

The reducing power of coffee samples was measured at 600 nm according to the procedure mentioned by Karawita et al. (2005), whereas, the chelating activity of ferrous ion by coffee samples was measured at 562 nm and was determined by the protocol of Koncic et al. (2011). To determine the ferrous ion chelating percentage, the following equation was used:

$$\% \text{ Chelation} = [(AB-AA)/AB] \times 100,$$

Where, AB: absorbance of blank sample and
 AA: absorbance of sample.

2.6 DPPH radical scavenging activity assay

To determine the free radical scavenging activity of coffee samples, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was used (Ohnishi et al., 1994). The absorbance was recorded at 517 nm and the inhibition percent equation of DPPH radical scavenging activity was expressed as:

$$\text{Percentage Scavenging} = [(AB-AA)/AB] \times 100,$$

Where, AB: absorbance of blank sample and
AA: absorbance of sample.

2.7 Scavenging of hydrogen peroxide assay

Hydrogen peroxide scavenging activity was performed according to the procedure of Gülçin et al. (2005) with some modifications. The absorbance was measured at 230 nm and the percentage of hydrogen peroxide scavenging activity by coffee samples was calculated by the following equation:

$$\text{Percentage Scavenging} = [(AB-AA)/AB] \times 100,$$

Where, AB: absorbance of blank sample and
AA: absorbance of sample.

2.8 Statistical analysis

The statistical analysis was performed using GraphPad Prism version 6.0. The data were analyzed by one-way analysis of variance (ANOVA), followed by a correction with Bonferroni's test. All the results were represented as mean $\pm$ SD from three independent experiments. Differences between groups were considered to be statistically significant at p value of <0.05 .

3 Results

3.1 Determination of the most consumed coffee type

The results obtained from the survey revealed that 55.4%, 23.8%, and 20.7% of studied participants consumed one, two, and three cups of coffee per day, respectively. Regarding the coffee type, more than 11 coffee types were included in the survey. Among these types, three coffee types showed a high percentage of consumption among the 900 participants. The most common coffee type consumed by the participants was Nescafé red mug coffee (25.2%) followed by Turkish coffee (16.4%) and finally, Espresso (8%).

3.2 Determination of total phenolic content

As determined by Folin Ciocalteu method, the total phenols content was reported as gallic acid equivalent to standard curve ($y=0.0022x$, $r^2=0.967$). The results for Nescafé red mug, Turkish coffee and Espresso were 741, 835 and 578 $\mu\text{g/ml}$ of gallic acid/2 g of coffee, respectively.

3.3 Determination of total flavonoid content

As determined by aluminum chloride method, the total flavonoid content was estimated as catechin equivalent to standard curve ($y=0.0025x$, $r^2=0.9213$). The results of study revealed the presence of 711, 802, and 828 $\mu\text{g/ml}$ of catechin/2 g of Nescafé red mug, Turkish coffee and Espresso coffee, respectively. The data revealed that there was no significant difference in the level of total flavonoid between the three coffee types.

3.4 Identification of ferric reducing antioxidant power and ferrous ion chelating activity

The presence of antioxidant, a reluctant substance in the experimental samples caused the reduction of the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) form. Therefore, by measuring the formation of Fe^{2+} , the reduction reaction can be monitored. In this assay, depending on the reducing power of antioxidant samples, the color of the test solution changed from yellow to green and blue. The reducing power capacity of a compound may serve as a significant signal of its potential antioxidant activity (Gülçin et al., 2010). The ferric reducing antioxidant power of Nescafé red mug, Turkish coffee and Espresso were 0.597, 0.428 and 0.832 mg/ml, respectively. The results showed that Espresso has a significantly ($p<0.01$) higher reducing power as compared to the other two coffee samples (figure1).

Figure 1 Ferric reducing power of Nescafé red mug, Turkish coffee and Espresso. The values were expressed as means $\pm$ SD ($n>3$). Comparison of means was calculated using One-way ANOVA followed by Bonferroni's correction test (** $p<0.01$).

Figure 2 Ferrous ion chelating activity of Nescafé red mug, Turkish coffee and Espresso. Espresso had significantly ($*p<0.05$) lower metal-ion chelating activity than Nescafé red mug and Turkish coffee. The values were expressed as means $\pm$ SD ($n>3$). Comparison of means was calculated using One-way ANOVA followed by Bonferroni's correction test ($*p<0.05$).

On the other hand, the ability of natural plant extracts to chelate transition metal ions can be resulted from the presence of phenolic compounds. These can prevent the formation of metal-induced free radical, by inhibiting the formation of complex between ferrous and ferrozine (Yusof et al., 2013). Ferrous ion chelating activity for Nescafé red mug coffee was 38.6 %, Turkish coffee was 50.7 % and Espresso was 22.9 %. Figure 2 showed that there were no significant differences in Fe²⁺ chelating activity in Turkish coffee when compared to Nescafé red mug, whereas, Espresso showed a significant decrease in Fe²⁺ chelating activity when compared to Nescafé red mug.

3.5 Determination of DPPH radical scavenging activity

The DPPH assay is a common method to determine the radical scavenging activity because it is reliable, does not require a special reaction and devices, easy and fast. The free radical scavenging activities of coffee depend on the structural conformation of antioxidant compounds and the ability of these compounds to lose hydrogen. The DPPH free radical, can receive easily an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule (Aksoy et al., 2013). The DPPH radical scavenging percent of Nescafé red mug, Turkish coffee and Espresso were 45.6 %, 28.7 % and 23.6 %, respectively. The results showed that there were significant decreases ($p<0.01$) in scavenging activity in Turkish coffee and Espresso compared to Nescafé red mug (figure 3).

Figure 3 DPPH radical scavenging activity of Nescafé red mug, Turkish coffee and Espresso. Turkish coffee and Espresso have significantly ($p<0.01$) lower radical scavenging activity compared to Nescafé red mug. Comparison of means was calculated using One-way ANOVA followed by Bonferroni's correction test ($**p<0.01$).

Figure 4 Hydrogen peroxide scavenging activity of Nescafé Red mug, Turkish coffee and Espresso. Turkish coffee had significantly lower (***) $p < 0.001$) scavenging activity for hydrogen peroxide, whereas Espresso had significantly higher (*) $p < 0.05$) scavenging activity for hydrogen peroxide compared to Nescafé red mug. Comparison of means was calculated using One-way ANOVA followed by Bonferroni's correction test (* $p < 0.05$ and *** $p < 0.001$).

3.6 Determination of hydrogen peroxide scavenging activity

Reactive oxygen species (ROS) include non-radical reactive derivatives and free radicals. Reactivity of non-radical species is generally weaker than free radicals. Hydrogen peroxide (H_2O_2) is an example of non-radical derivatives. Free radicals are unstable because they contain one or more electrons unpaired in their outer shell. Therefore, they tend to become stable either by accepting or by donating an electron (Priyanka et al., 2013). The scavenging percent of hydrogen peroxide of Nescafé red mug, Turkish coffee and Espresso were 77.8%, 27.2% and 87.2%, respectively, indicating that Turkish coffee had a highly significant reduction ($p < 0.001$) in H_2O_2 scavenging activity compared to the two other tested coffee types (figure 4).

4 Discussion

In the last decade, there has been a lot of attention to polyphenols, owing to their antioxidant capacity, metal chelating and free radical scavenging, and their possible beneficial effects on human health (Wollgast & Anklam, 2000). In plants, most of the antioxidant potential is due to the redox properties of phenolic compounds, which act as hydrogen donors, singlet oxygen quenchers and reducing agents (Kasote et al., 2015). Through various mechanisms, antioxidant activity of polyphenols may act as reducing, chelating and scavenging agent; act as cofactors of enzymes catalyzing oxidative reactions, terminate radical chain reactions, inhibit oxidases, and stabilize free radicals (Bogucka-Kocka et al., 2016). Maillard reaction is responsible for the generation of compounds called melanoidins, brown pigments in roasted coffee, from reducing amino acids and proteins or sugars during food processing and preservation (Wang et al., 2011; Karamac et al., 2005). Thermal processing

can induce the formation of new compounds with antioxidant properties or improve the properties of naturally occurring antioxidant. Therefore, the overall antioxidant activity remains unchanged or increased (Somporn et al., 2011).

Moreover, the antioxidant activity of coffee is related to the presence of important compounds such as chlorogenic, caffeic, ferulic, and *n*-coumaric acids. In some researches, caffeine was considered to be an important antioxidant agent (Karamac et al., 2005). According to the conducted survey, the results of the current study showed that Nescafé red mug, Turkish coffee and Espresso were the most consumed coffee types in Jeddah city, Western region of Saudi Arabia. Furthermore, there were no significant differences in reducing power activity between Nescafé red mug and Turkish coffee while Espresso had a higher ferric reducing power activity. This higher activity of Espresso to reduce ferric ion might be due to the high chlorogenic acid content. Indeed, a previous study supported a strong relationship between ferric reducing power activity and chlorogenic acid content (Moreira et al., 2005).

Regarding the scavenging activity, results of present study revealed that the highest radical scavenging activity was in Nescafé red mug and it was followed by Turkish coffee, whereas, the lowest scavenging activity was found in Espresso. Reduction in radical scavenging activity might be due to the roasting degree, and as well known the antiradical efficiency can be degraded during roasting (Erdem et al., 2016). Indeed, previous researches have reported that roasting degree can influence the total phenolic content and radical scavenging activity of coffee beans. The dark roasted coffee beans had lower radical scavenging than light roasted coffee beans (Del Castillo et al., 2002; Shan et al., 2015). Moreover, results of study revealed that the DPPH radical scavenging activity express lowest among the other types. It was previously

reported that the concentrations of caffeic acid and p-hydroxybenzoic acid seemed to be high in medium roasting coffee such as Nescafé red mug and Turkish coffee. In DPPH radical scavenging assay, hydroxybenzoic acid acts as a weak scavenger of DPPH radical (Somporn et al., 2011). Furthermore, results from hydrogen peroxide scavenging experiments showed a significant increase in both Espresso and in Nescafé red mug and a significant decrease in the Turkish coffee sample. The concentrations of ferulic acid, vanillic acid and protocatechuic acid, tend to increase when roasted in higher degrees such as Espresso (Shan et al., 2015). Vanillic acid is considered as one of the most active components in scavenging hydrogen peroxide. Hydroxybenzoic acid, which is present in Turkish coffee, showed a moderate antioxidant scavenging activity of hydrogen peroxide. Hydroxybenzoic acid is a weak scavenger of hydrogen peroxide (Somporn et al., 2011). Thus, better peroxyl radical scavenging activity is exerted in roasted dark coffee than the less roasted ones (Sánchez-González et al., 2005).

In addition, in this study, Nescafé red mug and Turkish coffee showed a higher chelating activity percentage than Espresso. hydroxycinnamic acid such as caffeic acid, which is high in Nescafé red mug and Turkish coffee, showed a better complex formation than protocatechuic acid, which is higher in Espresso coffee. The capacity of complex formation by hydroxycinnamic acid is relatively better than protocatechuic acid. Complex formation does not occur by all phenolic compounds, some of them are not metal chelators. For instance, phenolic acids, which are bearing catechol or gallyol group, such a vanillic acid and ferulic acid, which are present in high percentage in Espresso coffee, do not form chelation complex. On the other hand, phenolic acids, which are bearing catechol or gallyol group such as caffeic acid, showed a strong chelation activity (Andjelkovic et al., 2006).

Conclusion

This study demonstrated that Nescafé red mug, Turkish coffee and Espresso, were the most consumed coffee types in Jeddah according to the conducted survey. These three types of coffee showed high phenolic contents as well as a high antioxidant activity. Further *in vivo* and *in vitro* researches should be performed to estimate the benefits of these coffee brews and their effects on human health.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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