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Effects of different ingredients on antioxidant and oxidant status of brewed roasted coffee

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ABSTRACT

It was aimed to evaluate the effects of different ingredients on total antioxidant status (TAS) and total oxidant status (TOS) of the brewed Brazil mild roasted coffee. Sugar, sucralose, butter, coconut-oil, animal and plant-based milk types were added and milk types&sweeteners were combined separately. TAS and TOS were measured and oxidative stress indice (OSI) was calculated. TAS value of coffee with whole milk was the highest among all coffee types. TOS values of coffee with soy milk, with soy milk+sugar and soy milk+sucralose were lower than all other coffee types. When compared to OSI values, coffee with soy milk, with soy milk+sugar and soy milk+sucralose had the lowest OSI values among all coffee types. Moreover, addition of coconut-oil to coffee samples increased the TOS and OSI values of coffees. While the TOS and OSI of the sugar and sucralose addition were found higher in comparison to plain coffee; TOS and OSI of the sucralose addition was lower than those sugar combinations. In conclusion, the OSI of the coffee may change depending on the alterations of chemical structures and nutritional matrices by the added ingredients. The addition of soy milk or sucralose instead of sugar or whole cow milk instead of oil-based ingredients could be a promising strategy of reducing the oxidative capacity.

Keywords: Coffee, Milk, Sugar, Antioxidant, Oxidant

Introduction

Coffee is one of the most popular beverages due to its aroma and potential health effects worldwide (Samoggia & Riedel, 2019). Coffee compounds such as caffeine, chlorogenic acids, trigonelline, tryptophan alkaloids, diterpenes and other secondary metabolites are mainly responsible for its health benefits (Hu, Wang, Zhang, & Qiu, 2019). To date, numerous studies have suggested that acute or habitual coffee consumption has been positively related with multiple health outcomes with its antioxidant, anti-inflammatory, anticancer and antidiabetic properties due to having such large bioactive chemical compounds (Poole et al., 2017).

Recently it has been hypothesized that coffee has a potential protective role of against oxidative stress mediators and associated diseases. Martini et al. (2016) reported that chronic consumption of coffee may increase some antioxidant biomarkers (glutathione levels) and reduce the levels of Deoxyribonucleic acid (DNA) damage (Daniela Martini et al., 2016). In another study, daily 400 mL coffee consumption for 8 weeks contained medium or high chlorogenic acids (CGAs) increased plasma antioxidant capacity without any detrimental effect on vascular function in healthy adults (Agudelo-Ochoa et al., 2016). On the other hand, receiving 3 or 5 cups of study coffee (freshly brewed Arabica coffee using a filter machine and drunk for 2 times/day as 1–2 cups or 2–3 cups) or control (water) for 8 weeks had no beneficial effect on either DNA or plasma lipid levels (Shaposhnikov et al., 2018). However, CGAs the main phenolic component of coffee is primarily responsible of health benefits and has been associated with reduction in oxidative stress and consequently inflammation and chronic disease prevention like cancer and diabetes. (Tajik, Tajik, Mack, & Enck, 2017). Furthermore, other brewed coffee components such as some types of advanced glycation end products (AGEs) and/or colonic metabolites may responsible to inhibit plenty of diseases caused by oxidative damage as well (Gómez-Ruiz, Leake, & Ames, 2007; Yanagimoto, Ochi, Lee, & Shibamoto, 2004).

Roasting, manufacturing processes and preparation methods can alter antioxidant and phenolic substances of coffee (del Castillo, Ames, & Gordon, 2002; Niseteo, Komes, Belščak-Cvitanović, Horžić, & Budeč, 2012). Coffee can be consumed either plain or with desired flavoring ingredients such as sweeteners and/or milk (Samoggia & Riedel, 2019). Nutritional value of the consumption trends of beverages are usually ignored in exploring the health effects of consumption habits. However, active substances of coffee can interact with additives and thus alterations might occur within its health benefits (Nakilcioğlu-Taş, 2018; Niseteo et al., 2012). Sugar addition to Turkish coffee while cooking is shown to reduce antioxidant capacity (Nakilcioğlu-Taş, 2018). *In vitro* gastrointestinal digestion model showed milk addition can alter the bioaccessibility of coffee phenolic compounds in addition with particular processing methods (Quan et al., 2020). Additionally, coffee added with milk that contains 7.1% of fat increased chlorogenic acid bioaccessibility upon *in vitro* digestion after using high-pressure homogenization (Alongi, Calligaris, & Anese, 2019) suggesting higher milk fat enhances the bioaccessibility. On the other hand, milk additive (25%) was found insignificant on coffee antioxidant status in another study (Dupas, Marsset-Baglieri, Ordonaud, Ducept, & Maillard, 2006). However, milk addition on coffee brews decreased antioxidant capacity and polyphenolic content of brews, which refers to potential interactions between polyphenols and milk nutrients (Niseteo et al., 2012). Furthermore, adding milk and sugar into decaffeinated coffee before a high glycaemic index meal may positively effect on postprandial glycaemic and insulinaemic responses in healthy adults (Tommy, Jennifer, Iris, Sit, & Louie, 2020).

Impact of additive ingredients while consuming coffee and its health implications is poorly understood and results are conflicting. Especially, new trends like adding butter or coconut oil into brewed coffee and their effects on oxidation status have not been studied before. Since having important antioxidant properties, coffee might provide beneficial effects on oxidative stress markers as well and hence, human health (Martini et al., 2016). Therefore, we aimed to evaluate the effects of different ingredients on antioxidant and oxidant properties of brewed roasted coffee in the present study.

Materials and Methods

Sample Selection and Brewing Conditions

Roasted coffee beans (Brazil mild, roasted to 156-165 °C) were supplied from a local coffee supplier. A coffee grinder (Russell Hobbs 23120-56, United Kingdom) were used to obtain grounded filter coffee. All samples were prepared with filter coffee machine in compliance with The Specialty Coffee Association's (2020) coffee cupping standards within 5 minutes. According to this standards brewing were done with the ratio of 8.25 ±0.25 grams (whole bean) coffee to 150 mL water. Water temperature was 92.2-94.4 °C when poured on grounds. Then, tempered glass cups were used for cupping.

Ingredients

It was prepared plain (black) filter coffee as described above and added sugar (5 g), sucralose (0.5 g), butter (5 g), coconut oil (5 g), ultra high temperature (UHT) cow milk types (20% v/v, ~30 mL); whole milk, light milk, lactose-free milk, and

UHT coconut milk and soy milk at room temperature. Additionally, milk types and sweeteners were combined separately. Amount of milk was decided based on earlier literature (Al-Ghafari et al., 2017; Dupas et al., 2006); while the others were taken as one teaspoon (sucralose taken as equaling to same amount of sucrose). All ingredients were purchased from the local markets. Characteristics and nutrition facts of added ingredients to coffee were given in Table 1. The final temperatures of samples with ingredients were shown in Table 2.

Sample Analyses

Brewed coffee was taken to tempered glass and added the ingredients. The final temperature of samples was measured. Then, coffee samples collected into micro tubes and analyzed immediately. All samples were analyzed in duplicate.

Measurement of Temperature

Water used for preparing coffee samples was heated using a heat adjustable kettle with glass chamber (Rossman™), and brewing temperatures were measured by a probe thermometer (Arcone TP101™).

Measurement of Water-Soluble Dry Matter Content

A portable ATC Brix refractometer was used to measure of dry matter of brewed coffee samples. Briefly, the zero-calibration using distilled water was performed. Then, a few drops of the sample were placed on the measurement prism. The cover plate was closed for spreading the liquid across the entire surface of the prism and avoided to produce any air bubbles or dry spots. While holding the instrument under a light source, the Brix concentration (◦) was determined by the intersection of the boundary of the light and dark fields on the scale. After reading, the instrument was cleaned with distilled water immediately.

Analysis of Total Antioxidant Status (TAS) and Total Oxidant Status (TOS)

Total antioxidant status (TAS) and total oxidant status (TOS) of coffee samples were analyzed using commercially available kits (Relassay, Turkey) and Mindray BS300 Auto Biochemistry Analyzer™ following the kit protocol. Coffee samples were centrifuged (Selecta Centronic BLII) at 3000 rpm for 2 minutes at 4℃. For the measurement of TAS values the novel automated method were used based on the bleaching of characteristic color of a more stable 2,2 Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation by antioxidants. The precision value of assay was lower than 3%. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The results were expressed as mmol Trolox equivalent/L (Ozcan Erel, 2004).

For the measurement of TOS values, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, measured by spectrophotometrically in 530 nm, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micro molar hydrogen peroxide equivalent per liter (mol H_2O_2 equivalent/L) (O. Erel, 2005).

Calculation of Oxidative Stress Index (OSI)

Oxidative stress index (OSI) were used as an indicator of oxidative stress (the ratio of TOS to TAS) calculated using the following formula (Yumru et al., 2009): OSI (arbitrary unit) =TOS (μmol H_2O_2 equivalent/L) / TAS (μmol Trolox equivalent/L).

Table 1. Characteristics and nutrition facts of added ingredients

Ingredients	Brand	Nutrition Facts (g/100 g)					
		Carbohydrate	Sugars	Fat	SFA	Protein	Fiber
Sugar	A	99.9	99.9	-	-	-	-
Sucralose	B	98.8	7.1	-	-	-	-
Butter	C	0.8	N/A	82	51.3	0.6	N/A
Coconut oil	D	-	-	100	93	-	N/A
Whole milk	C	4.5	4.5	3.3	2.1	3.0	N/A
Light milk	C	5.0	5.0	0.1	-	3.1	N/A
Lactose-free milk	C	4.7	4.7	1.5	0.9	3.0	N/A
Coconut milk	E	2.7	1.9	0.9	0.9	0.1	0.1
Soy milk	E	2.5	2.5	1.8	0.3	3.0	0.5

SFA: Saturated fatty acids, N/A: Not available

Statistical Analysis

All data were analyzed using SPSS 22.0 statistical package program. Arithmetic mean and standard deviation values ($\bar{x}$ ±SD) are given as descriptive statistics for variables. Kruskal-Wallis test was to determine differences among TAS, TOS and OSI values of different coffee samples. Moreover, pair-wise comparisons of OSI values among coffee types were shown with Mann-Whitney U test. Statistically significance level was accepted as $p<0.05$ for all analyses.

Results and Discussion

Coffee is one the most consumed beverages on a global scale due to its flavor and certain potential effects (Wang & Ho, 2009). Many people add some ingredients to coffee instead of consuming it plain in order to obtain a smooth taste or to increase its flavor and health contributions (Al-Ghafari et al., 2017). However, information about the effects of the added ingredients on coffee, which is known for its polyphenol and antioxidant capacity, is limited, and research in this regard still continues. On this context, the study was conducted in order to investigate the effects of adding ingredients with very different chemical properties and matrices on their own and/or in combination on the antioxidant/oxidant status of coffee in a wide range.

Table 2. Temperature (°C), dry matter content ($Brix^0$), TAS (mmol/L), TOS (µmol/L) and OSI values of roasted coffee with added ingredients brewed with filter coffee machine

Ingredients and quantity	Temperature (°C)	Dry matter ($Brix^o$)	TAS (mmol/L)	TOS (µmol/L)	OSI
Black (just brewed coffee)	61.8	1.336	2.97±0.00	49.11±7.29	1.65±0.25
Sweeteners					
Sugar (5 g)	46.8	1.349	2.98±0.01	59.97±2.42	2.02±0.08
Sucralose (0.5 g)	48.2	1.336	2.97±0.01	52.38±1.01	1.77±0.03
Fats and Oils					
Butter (5 g)	42.7	1.336	2.98±0.00	48.33±6.16	1.62±0.21
Coconut oil (5 g)	41.1	N/A	2.96±0.01	55.54±1.50	1.87±0.05
Milks					
Whole milk (30 mL)	42.9	1.342	3.12±0.04	36.32±3.69	1.16±0.10
Light milk (30 mL)	39.1	1.338	3.03±0.01	34.96±3.93	1.16±0.13
Lactose free milk (30 mL)	42.8	1.340	3.04±0.00	30.24±1.09	0.99±0.04
Coconut milk (30 mL)	39.5	1.337	3.00±0.01	42.99±4.59	1.43±0.15
Soy milk (30 mL)	40.8	1.338	2.92±0.01	6.58±0.27	0.23±0.01
Milk with sweeteners					
Whole milk (30 mL) with sugar (5 g)	39.4	1.353	3.16±0.01	38.48±0.31	1.22±0.01
Light milk (30 mL) with sugar (5 g)	41.4	1.353	3.04±0.02	36.14±3.41	1.19±0.12
Lactose free milk (30 mL) with sugar (5 g)	40.3	1.350	3.04±0.00	36.51±2.62	1.20±0.09
Coconut milk (30 mL) with sugar (5 g)	39.8	1.347	3.02±0.02	46.54±3.36	1.54±0.10
Soy milk (30 mL) with sugar (5 g)	39.3	1.347	2.92±0.01	6.41±0.61	0.22±0.02
Whole milk (30 mL) with sucralose (0.5 g)	41.1	1.341	3.09±0.06	33.80±1.59	1.09±0.03
Light milk (30 mL) with sucralose (0.5 g)	41.1	1.339	3.04±0.02	36.78±2.70	1.21±0.10
Lactose free milk (30 mL) with sucralose (0.5 g)	42.8	1.340	3.04±0.00	34.92±0.01	1.15±0.00
Coconut milk (30 mL) with sucralose (0.5 g)	43.5	1.336	3.02±0.03	39.56±0.79	1.31±0.01
Soy milk (30 mL) with sucralose (0.5 g)	40.5	1.339	2.88±0.00	4.62±0.59	0.16±0.02
p value*			***p=0.000***	***p=0.000***	***p=0.000***

Dry matter: Water soluble dry matter content, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, N/A: Not available

*Kruskal Wallis test

In the present study, TAS value of plain/black Brazilian mild roasted coffee brewed with filter coffee machine was determined to be 2.97 ±0.00 mmol/L (Table 2). Before it is roasted, the green coffee bean has a complex matrix of carbohydrate (~60% in dry substance), lipid (8%–18% in dry substance), protein, peptide and free amino acid (9%–16% in dry substance), caffeine and trigonelline, theobromine, theophylline. It is reported that coffee bean contains 6-10% polyphenol in dry substance, that the most dominant of these polyphenols are chlorogenic acids (CGAs) – including the caffeoylquinic acids (CQAs), especially 5-CQA, and that it contains feruloylquinic acids and dicaffeoylquinic acids in less amounts (Acar-Tek, Ağagündüz, & Ayhan, 2018). It has also been reported that coffee polyphenols, especially chlorogenic acid, have health improving anti-inflammatory, anticarcinogenic, antidiabetic, and antihypertension effects. Besides, there is evidence that chlorogenic acid protects vascular endothelial cells and reduces metabolic syndrome risk by increasing endothelial nitric oxide synthase expression and nitric oxide generation with its antioxidant properties (Yamagata, 2018). Although partial reductions and/or transformations may occur in these components following the roasting of coffee beans (180-250 °C), coffee continues to show its antioxidant activity (Acar-Tek et al., 2018; Richelle, Tavazzi, & Offord, 2001) In a study in which the effects of the production and roasting conditions of coffee on the antioxidant effects of coffee brews were investigated, it was reported that especially light and medium roasted coffee brews had the highest antioxidant activity compared to dark coffee, and that they might protect cells from oxidative stress damages (Duarte, Abreu, Menezes, Santos, & Gouvêa, 2005). Similarly, as it was aimed to examine the antioxidant activities of coffee brews in this study, medium roasting method was employed. Furthermore, when antioxidant activity and bioactive substances are handled, extraction mechanisms in obtaining coffee brews play an important role as well (Caprioli, Cortese, Sagratini, & Vittori, 2015). Filtering method is one of the leading extraction techniques in terms of the antioxidant activity of coffee. In a study that supports this, the antioxidant capacities of the aqueous spent coffee extracts were determined as 46.0-102.3% (filter), 59.2-85.6% (espresso), and <42% (plunger) (Bravo et al., 2012). The results of this study reflect the results of brewing method using filter coffee machine.

One of the most significant findings of this study was that the added milk types could change the antioxidant/oxidant capacity of coffee in different ways depending on the fractions and sources/types of milk (Table 2, Figure 1). In the study, it was determined that the antioxidant activities of the coffee types (with/without sucralose) to which various cow milk fractions (whole, light, lactose-free), especially whole cow milk (20%), were added were significantly higher compared to other coffee types, including black coffee (Table 2). Among the ingredients that can be added to coffee, especially milk is one of the leading ingredients due to its nutritional composition (protein, fat, sugars and vitamins-minerals). Milk is a food product that contains many essential nutrients as well as many components with antioxidant effects. Protein fractions in milk, especially casein, show antioxidant activity (Fardet & Rock, 2018). Antioxidant enzymes in milk, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and conjugated linoleic acid, coenzyme Q10, vitamins C, E, A and D3, equol, uric acid, carotenoids, and minerals contribute to the antioxidant activity of milk (Fardet & Rock, 2018; Khan et al., 2019). However, when milk is added to coffee, milk proteins such as whey and casein interact with coffee polyphenols by forming a complex. This condition may have an effect on functional and nutritional properties of coffee (Al-Ghafari et al., 2017). Similar to this study, in a study, it was determined that milk added to coffee by 10% and 20% enhanced the scavenging of 2,2-diphenyl-1-pycrylhydrazyl (DPPH) or decreased the metal chelating and metal reducing activity of polyphenol (Al-Ghafari et al., 2017). In contrast to this study, it was determined that adding milk to instant coffee brews decreased the total phenol and chlorogenic acid variety components, and ABTS and ferric-reducing antioxidant power (FRAP) antioxidant activities (Niseteo et al., 2012). Even in a study, addition of fresh whole milk or evaporated milk affected antioxidant activity through different mechanisms due to solid substance ingredients (fat and protein) (Alsufiani, 2017). In another study conducted, it was determined that when milk was added to coffee by 25%, approximately 40% of chlorogenic acid in coffee was in a bonded condition and its bioavailability decreased, but that addition of milk did not have a significant effect on coffee's DPPH and 2-2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-antioxidant effect (Dupas et al., 2006). As issues such as the type of coffee and the amount of added milk mostly are not clearly stated, and a standard evaluation method is not used, it becomes difficult to evaluate the studies. Also, the studies conducted in the literature mostly focused on how the milk added to coffee changed the bioavailability of coffee (Duarte & Farah, 2011; Quan et al., 2020; Renouf et al., 2010). In a study conducted, it was reported that addition of whole milk did not change the overall bioavailability of coffee polyphenols, but that sugar and non-dairy creamer affected the maximum plasma concentration-C(max) of coffee polyphenols and the time needed to reach C(max)-T(max) (Renouf et al., 2010). In another study, consumption of coffee and milk together was determined to decrease the bioavailability of chlorogenic acid and metabolites (40% vs 68%) compared to consuming coffee plain (Duarte & Farah, 2011). In yet another study, the effect of addition of milk in

different matrices (skimmed milk and whole milk) on antioxidant capacity in in vitro gastrointestinal digestion model and the bio-accessibility of phenolics was investigated, and following the additions of milk, the antioxidant capacity of all samples were reduced or did not change. However, it was determined that especially the addition of whole milk displayed better phenolic bio-accessibility compared to skimmed milk (Quan et al., 2020). These results shed light on the necessity in future studies for a focus not only on nutritional matrix and capacity changes, but also on how it changes bioavailability and antioxidant capacity in different models.

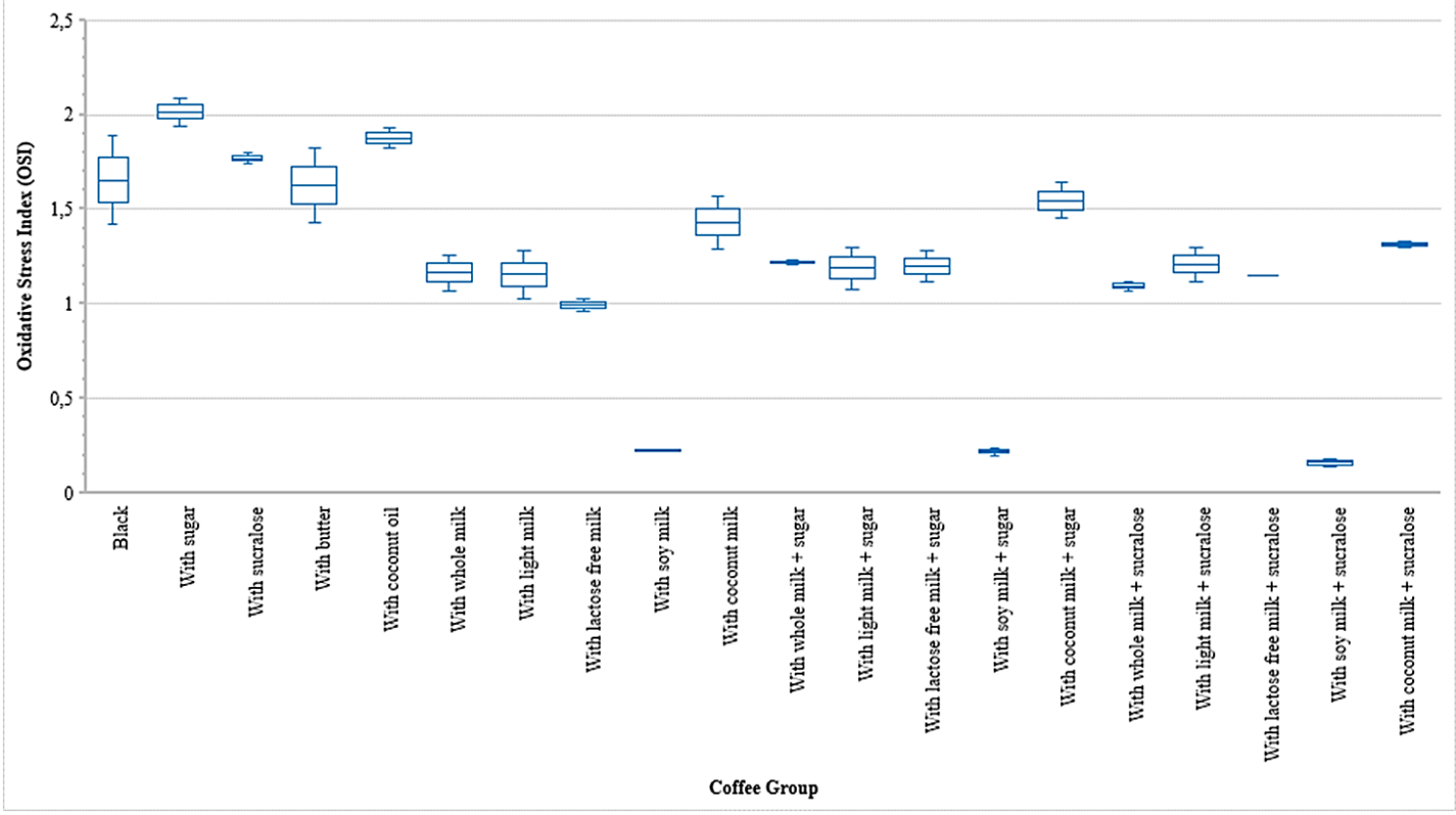

Figure 1. The OSI values of coffee samples with different ingredients. Pairwise comparisons of coffee groups according to OSI (Mann Whitney U test, $p<0.05$): 1) OSI of the coffee "with whole milk" was statistically lower than OSI of the "black coffee", "coffee with sugar", "with sucralose", "with butter" and "with coconut oil". 2) OSI of the coffee "with light milk" was statistically lower than OSI of the "coffee with sugar" and "with coconut oil". 3) OSI of the coffee "with lactose-free milk" was statistically lower than OSI of the "black coffee", "coffee with sugar", "with sucralose", "with butter", "with coconut oil", "with coconut milk" and with coconut milk+sugar". 4) OSI of the coffee "with soy milk", "with soy milk+sugar" and "with soy milk+sucralose" were statistically lower than OSI of the "black coffee", "coffee with sugar", "with sucralose", "with butter", "with coconut oil", "with coconut milk", with coconut milk+sugar" and "with coconut milk+sucralose

Reactive oxygen species (ROS) are formed during cellular metabolism of macronutrient elements in energy production. As they are highly reactive molecules, lipids cause damage to biological structures such as proteins, polysaccharides, and DNA. Under normal physiological conditions, antioxidants can block the harmful effects of ROS, but in case of an imbalance between ROS and antioxidant defense mechanisms, oxidative stress can occur (Kalyanaraman, 2013). One of the important findings of this study was that the added ingredients could change oxidant activity/oxidative stress rather than antioxidant activity (Table 2, Figure 1). TOS value and OSI value of plain/black roasted coffee brewed with filter coffee machines was found to be 49.11 ±7.29 μmol/L and 1.65 ±0.25, respectively, and especially addition of soy milk from plant-based milk types (sucralose and sugar combinations included) decreased these values significantly. Soybean and soy products have a matrix composed of bioactive components such as saponins, protease inhibitors, phytic acid and isoflavones (Barrett, 2006). Soy bean stands out with its high

isoflavones content (McCue, Horii, & Shetty, 2004). Isoflavones, also known as phytoestrogens, are compounds with estrogen-like structures (Barrett, 2006). Polyphenols are mostly found in the form of glucosides (daidzin and genistin) and aglycones (daidzein and genistein), and the antioxidant activity of soy bonds with these compounds (McCue et al., 2004). In a similar study to the present study, the antioxidant capability of UHT soy milk was found to be higher compared to normal UHT milk, and it was claimed that these antioxidant properties could not be explained only by phenolic compounds, and that peptides and amino-acids in soy milk resulting from the UHT production process could also display antioxidant properties (Baghbadorani et al., 2017). In another study conducted, it was reported that adding soy milk to tea types increased and/or did not change total antioxidant activity, but that it could be a better alternative in terms of antioxidant activity compared to bovine milk (Ryan & Sutherland, 2011). It was reported that when green coffee extract was enhanced with soy milk (0.025-1 mg), phenolic substance content increased up to 70 %, and that the antiradical activity and reducing power increased by 2 to 10 times (Sęczyk, Świeca, & Gawlik-Dziki, 2017). In another study carried out, consumption of probiotic soymilk with the dosage of 200 mL per day by Type 2 diabetic kidney patients improved their oxidative stress markers (Miraghajani, Zaghian, Mirlohi, Feizi, & Ghiasvand, 2017). As reported in other studies as well, it is thought that the antioxidant content and activity of soy is the main reason for the positive effect of soymilk on oxidative stress in this study.

In the present study, another finding was that the oxidant status and oxidative stress of coffee samples added with sugar and sucralose (single or in combination) were found to be higher in comparison to coffee samples with no addition (Table 2, Figure 1). In a similar study conducted on Turkish-style coffee brews, it was determined that light roasted coffee had higher polyphenol content, but that medium roasted coffee brews showed higher antioxidant capacity, and that especially those without sugar provided more health benefits (Nakilcioğlu-Taş, 2018). On the other hand, in another study, addition of sugar, milk, and lemon juice to green tea was determined not to change the antioxidant capacity of tea infusions significantly (Bartoszek, Polak, & Chorążewski, 2018). Again, in a study on black tea, it was observed that radical scavenging activity of black tea was the highest, followed by black tea + sugar and black tea + milk+sugar (Sharma, Vijay Kumar, & Jagan Mohan Rao, 2008). Even in a study, it was determined that alternative sweeteners to refined sugar such as dark and blackstrap molasses (FRAP: 4.6 to 4.9 mmol/100g), maple syrup, brown sugar, and honey (FRAP: 0.2 to 0.7 mmol/100g) had higher antioxidant activities (Phillips, Carlsen, & Blomhoff, 2009). In this study too, it was determined that the use of sucralose, which is a non-nutritive, zero-calorie artificial sweetener, instead of sugar could be a better alternative in terms of oxidative status and stress (Table 2, Figure 1). Sucralose, 600 times sweeter than sugar, is a chlorinated (three chlorine atoms replace three hydroxyl groups) sugar substitute approved by the U.S. Food and Drug Administration (AlDeeb, Mahgoub, & Foda, 2013). Although there is an accumulated literature regarding the use of artificial sweeteners like sucralose in the struggle against obesity (Khan, 2015), there is almost no information on its potential antioxidant/oxidative effects. Though the health effects of these sweeteners are still being discussed, it was found in this study that they had more promising effects in terms of oxidative status in comparison to common table sugar. This situation can be associated with the fact that consumers put less amounts of sucralose than common table sugar equivalent to the taste of sugar in order to consume their coffee for sensorial reasons.

In this study, the oxidant status and oxidative stress of especially coffee samples with coconut oil addition (single or in combination) were found to be higher (Table 2, Figure 1). Coconut oil is an edible oil that is extracted from the kernel of mature coconuts harvested from the coconut palm. Coconut oil has an increasing popularity recently due to its bioactive substance and particularly medium-chain fatty acids content (Wallace, 2019). However, there are contradictory findings regarding its effects on health and nutrition, especially on blood lipids (Boateng, Ansong, Owusu, & Steiner-Asiedu, 2016). In the literature, it is stated that coconut oil intake should not exceed 10% of total caloric intake, especially due to its saturated fatty acid content and cardiometabolic profile. It was also determined in the same study that its addition to coffee samples was not a good strategy in terms of oxidative status (Santos, Howell, Earnest, & Teixeira, 2019). For those who are fond of its flavor, coconut milk consumption could be a better choice instead of consuming oil fraction.

Conclusion

In conclusion, in this study, in which the effect of ingredients with different chemical structure and nutritional matrix on the antioxidant and oxidant status of brewed coffee was investigated and it was determined that mostly the oxidant status/oxidative stress of coffee rather than its antioxidant capacity could change depending on the added ingredients.

There are some limitations in this study. The first of these limitations is that characterization of nutrients and bioactive substances which may affect the antioxidant/oxidant capacity of coffee samples was not conducted in this study. Secondly, all antioxidant/oxidant capacity values of coffee samples added ingredients found in this study could not be compared

since there was not any similar study related to some ingredients in the literature. Thirdly, this study only reflects the results of the filtered- Brazilian mild roasted coffee. Therefore, it is thought that the results obtained in this study may be partially limited in the generalization of the all coffee types using different beans, extraction and preparation methods. It is recommended to consider these conditions in future studies.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

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