

Antioxidant Activity and Phytochemicals of Freeze-dried and Spray-dried Soluble Coffee Brews

I Dewa Ayu Susilawati^{1*} , Fredi Muzeka² 

¹Coffee for Social Welfare Research Group Universitas Jember; Department of Biomedical Science, Faculty of Dentistry, Universitas Jember, Indonesia

²Healthcare professionals of Nahdatul Ulama Sayyid Abdurrahman Clinic, Jombang, Indonesia

*Corresponding author: dewasusi@unej.ac.id

Editor: Samuel Pereira de Carvalho

Received in January 24, 2025 and approved in March 18, 2025

ABSTRACT

This study analyzed the antioxidant activity and the concentration of several phytochemicals i.e. polyphenols, caffeine, glutathione (GSH), and protein, of freeze-dried and spray-dried soluble coffee brews. Six types of coffee samples were analyzed i.e., 1) Spray-Dried Robusta instant coffee (SDR), 2) Freeze-Dried Robusta instant coffee (FDR), 3) Regular Robusta coffee powder (RR), 4) Spray-Dried Arabica instant coffee (SDA), 5) Freeze-Dried Arabica instant coffee (FDA), and 6) Regular Arabica coffee powder (RA). Antioxidant activity was determined using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay. Polyphenols, caffeine, glutathione, and protein were measured spectrophotometrically. Results showed that freeze-dried instant coffee (both robusta and arabica) in a concentration of 500 ppm demonstrated DPPH radical scavenger activity >80% and significantly higher ($p < 0.05$) compared to spray-dried soluble coffee and regular coffee brews. This level was maintained in concentrations of 750 ppm, 1000 ppm, and 1500 ppm. The higher antioxidant activity of freeze-dried coffee brew was in line with the higher concentration of polyphenols, caffeine, glutathione, and protein. In conclusion, freeze-dried instant coffee demonstrated more sensitive antioxidant activity, probably due to its more abundant polyphenols, caffeine, glutathione, and protein.

Key words: Caffeine; DPPH; Glutathione; Polyphenols; Protein.

1 INTRODUCTION

The coffee brew is a healthy drink (Grosso et al., 2017; Poole et al., 2017). Coffee consumption within usual levels is associated with a longer lifespan and reduces the risk of death from various diseases including heart disease, stroke, cancer, and diabetes (Crippa et al. 2014; Gunter et al. 2017). Drinking coffee enhances neurocognitive function (Kim; Kang; Kim, 2021). The health benefit of coffee was suggested due to its phytochemicals, particularly the antioxidant compounds (Dludla et al., 2023).

Antioxidants are crucial for scavenging free radicals and oxidants and reducing oxidative stress in the body (Martini et al., 2016; Yashin et al., 2013). Coffee is one of the best exogenous sources of antioxidants such as flavonoids and phenolic acids (Górecki; Hallmann, 2020; Alnsour et al., 2022). The concentration of antioxidant phytochemicals influences the antioxidant activity of coffee. In addition to phenolic compounds, proteins and glutathione (GSH) from food have excellent potential as antioxidants (Prodić et al., 2023; Di Giacomo et al., 2023). Only a few studies, however, reported the role of protein and glutathione in coffee as antioxidants.

Coffee brews can be made from regular or soluble (instant) coffee grounds/powder. The regular coffee grounds did not dissolve completely in the water, and remained as spent grounds, while instant coffee grounds dissolved instantly. Generally, there are two ways to make instant coffee, i.e. spray-drying and freeze-drying. The freeze-drying process

was conducted at sub-zero temperatures and preserved the phytochemicals (Barroso et al., 2024; Marcucci et al., 2017). In comparison, the spray-drying process conducted at high temperatures might result in the degradation of phytochemicals and the reduction of antioxidant activity. This study analyzed the antioxidant activity and the concentration of several phytochemicals, i.e. polyphenols, caffeine, glutathione (GSH), and protein of freeze-dried and spray-dried soluble coffee brews, and compared them to regular coffee brews.

2 MATERIAL AND METHODS

There were six types of samples: 1) spray-dried Robusta instant coffee (SDR), 2) freeze-dried Robusta instant coffee (FDR), 3) Regular Robusta coffee powder (RR), 4) spray-dried Arabica instant coffee (SDA), 5) freeze-dried Arabica instant coffee (FDA), and 6) Regular Arabica coffee powder (RA). The commercial regular coffee powder (Robusta and Arabica) was purchased from the same factory (State Coffee Plantation PTPN XII Indonesia). Coffee spray-drying was processed using LabPlant SD-06 Spray Dryer and freeze-drying using equipment, such as a vacuum freeze dryer (Toption TPV-50F). Reagents for DPPH (1,1-diphenyl 2-picrylhydrazyl), protein, and phytochemical analysis were purchased from Sigma, and Dichloromethane from Aldrich, Germany. The glutathione assay kit was QuantiChrom™.

Preparation of spray-dried instant coffee. The powder of this instant coffee was prepared from the commercial regular

coffee powder. The extract of regular coffee powder was sprayed into hot air to form tiny droplets. Then, the heat dries and turns them into fine powder or small pieces, according to the method by Varelzis et al. (2020), with some modifications. Briefly, 250 g regular coffee powder (Robusta or Arabica) was brewed in 2 L boiled distilled water, stirred using an ultrasonic stirrer for five minutes, and then filtered (100 Mesh). The filtrate was then sprayed into the spray-dried apparatus, at the temperature inlet 120 °C and outlet 60 °C. The fine powder was then collected and preserved in a sterile plastic bag and kept at room temperature until before being analyzed. This process yielded two kinds of samples, i.e., spray dried Robusta instant coffee (SDR) and spray-dried Arabica instant coffee (SDA).

Preparation of freeze-dried instant coffee. The powder of its instant coffee was prepared from commercial regular coffee powder (the same sample for making SD instant coffee). Principally, the extract of regular coffee powder was frozen and then dried at a low temperature in a vacuum according to the method by Varelzis et al. (2020), with some modifications. A total of 250 g regular coffee powder (Robusta or Arabica) was brewed in 2 L boiled distilled water, stirred using an ultrasonic stirrer for five minutes, and then filtered (100 Mesh). The filtrate was frozen at minus temperature (-50°C) for 48 h until a fine powder was formed. This procedure yielded samples of freeze-dried Robusta instant coffee (FDR) and freeze-dried Arabica instant coffee (FDA).

Preparation of coffee brew. The coffee brew solution for analysis was expressed in parts per million (ppm). To prepare a 1000 ppm coffee solution, 10 mg of coffee powder was dissolved in 10 ml of distilled water, stirred, and filtered using Whatman filter paper. The filtrate of each sample was prepared in a working serial concentration, i.e., 100 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm, and 1500 ppm, and ready for analysis using the DPPH method. Each sample concentration was prepared in a triplet.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was assayed according to the method described by Mokoroane et al. (2020) with some modifications. Each sample of coffee solution was mixed with ethanolic DPPH solution (100 µl coffee solution, 400 µl ethanol, and 500 µl DPPH) and shaken vigorously. The solutions were then allowed for 30 min at room temperature under dark conditions. The DPPH radical scavenging activity was indicated by the color change of the solution from purple to yellow. All samples were tested for their absorbance (A) using a UV-visible spectrophotometer at a wavelength of 514 nm, and performed in triplicate. The value of absorbance was determined using ascorbic acid as a reference. The DPPH radical scavenging activity was expressed as the percentage of DPPH radical inhibition and calculated using the formula: $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100\%$.

The concentration of polyphenol was determined using the Folin-Ciocalteu reagent and gallic acid as a reference

standard, according to a method described by Al-Owaisi et al. (2014) with some modifications. A 50 µL sample was added with 4950 µL distilled water, mixed with 0.5 mL Folin-Ciocalteu reagent, vortexed, and incubated for 5 min. The mixture was then added with 1 mL sodium carbonate (70%), vortexed, and allowed in the dark at room temperature for 60 min with intermittent shaking. The absorbance was measured at 765 nm by a UV-VIS spectrophotometer, using gallic acid as standard. All measurements were performed in duplicate.

Caffeine concentration in coffee was measured using distilled water and dichloromethane, according to the method by Belay et al. (2008) with some modifications. A sample of 0.05 g coffee ground was dissolved in 10 mL distilled water, then heated at 90°C for 10 min, cooled until it reached room temperature, and filtered. The filtrate was placed into a glass separatory funnel, added with 25 mL dichloromethane and shaken for 3 min. This procedure separated dichloromethane into the bottom layer and drained into a clean container. This separation procedure was repeated four times so that a total of 100 mL of dichloromethane was used. The absorbance of the solution was measured by a UV-VIS Spectrophotometer at a wavelength of 285 nm.

The concentration of GSH in coffee brews was determined according to the available protocol of the Glutathione assay kit. A sample volume of 400 µL was mixed with 400 µL of reagent A. Then 600 µL supernatant was mixed with 400 µL reagent B, incubated for 25 min at room temperature, and the optical density was measured at 412 nm against water. The concentration was expressed as mg/dL.

The protein concentration in coffee brews was determined using the Bradford protein assay (according to the available protocol). An amount sample of 1 g of coffee powder was dissolved in 15 mL of boiled distilled water, stirred for 5 min, and left to reach room temperature. Then, 5 µL supernatant was taken and 995 µL Bradford reagent was added, after 10-15 min, the solution was then analyzed using a UV-VIS Spectrophotometer, and the absorbance was measured at wavelength 595 nm.

Data on the percentage of DPPH radical inhibition was expressed as the mean $\pm$ standard deviation (mean $\pm$ SD) of the triplicate samples, while the phytochemical of the duplicate samples. Data were analyzed descriptively and statistically using one-way analysis of variance (ANOVA).

3 RESULTS

Freeze-dried soluble coffee showed a significantly higher ($p < 0.05$) DPPH radical scavenging activity compared the regular coffee, almost in all studied concentrations. Freeze-dried soluble coffee both Robusta and Arabica demonstrated radical scavenging activity $> 80\%$ in the concentration of 500 ppm, and this level was maintained in concentrations of 750 ppm, 1000 ppm, and 1500 ppm. Interestingly, all samples

demonstrated the DPPH radical scavenging activity >80% in the concentration 1500 ppm; statistically, there was no significant difference ($p>0.05$). Measurements of the DPPH radical scavenging activity of six types of coffee brews were presented in Table 1 and Figure 1.

Phytochemical analysis showed that freeze-dried both arabica and robusta coffee contained higher polyphenols, caffeine, and protein compared to spray-dried and regular coffee. Interestingly, the concentration of glutathione in arabica coffee (regular and freeze-dried) was higher compared to robusta coffee (Table 2).

Table 1: The DPPH Radical Scavenging Activity of Regular and Soluble Coffee Brews.

ppm	Coffee Brews Scavenging Activity Against DPPH Radical (% inhibition)					
	Regular Coffee		Soluble coffee			
			Freeze-dried		Spray-dried	
	RR	RA	FDR	FDA	SDR	SDA
100	1.69±1.50	7.36±0.34 ⁿ¹	29.77±0.71	16.18±0.37 ⁿ²	8.32±1.10 ⁿ¹	15.36±3.93 ⁿ²
250	13.85±1.58	26.84±0.87	66.71±0.65	59.67±0.92	34.75±2.36	38.91±1.27
500	33.88±0.83	54.36±1.76	87.19±1.96	82.76±1.16	61.22±0.89	69.63±6.09
750	54.36±0.55	72.19±1.24	83.50±0.15 ⁿ³	84.46±0.37 ⁿ³	78.09±2.23	85.09±1.58 ⁿ³
1000	72.51±1.95	83.12±0.84 ⁿ⁴	84.72±1.05 ⁿ⁴	84.07±0.52 ⁿ⁴	77.22±1.48	82.71±1.81 ⁿ⁴
1500	82.99±3.33 ⁿ⁵	83.40±0.84 ⁿ⁵	83.81±2.21 ⁿ⁵	84.46±0.30 ⁿ⁵	85.32±1.07 ⁿ⁵	84.72±2.00 ⁿ⁵

n: no significant difference ($p>0.05$) between samples in each concentration (equal superscript).

RR: Regular Robusta Coffee.

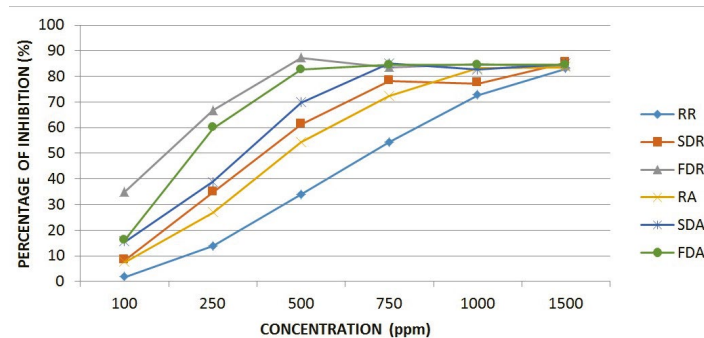
RA: Regular Arabica Coffee.

SDR: Spray-dried Robusta Soluble Coffee.

SDA: Spray-dried Arabica Soluble Coffee.

FDR: Freeze-dried Robusta Soluble Coffee.

FDA: Freeze-dried Arabica Soluble Coffee.



RR: Regular Robusta Coffee

RA: Regular Arabica Coffee

SDR: Spray-dried Robusta Soluble Coffee

SDA: Spray-dried Arabica Soluble Coffee

FDR: Freeze-dried Robusta Soluble Coffee

FDA: Freeze-dried Arabica Soluble Coffee

Figure 1: Coffee brew scavenging activity against the DPPH radical.

Table 2: The phytochemicals concentration of coffee brews.

Compounds	Coffee Brews Phytochemicals Concentration					
	Regular Coffee		Soluble Coffee			
	RR	RA	Freeze-dried		Spray-dried	
			FDR	FDA	SDR	SDA
Polyphenols (mg/g)	16.25	16.05	75.23	75.57	29.59	25.97
Caffeine (%)	2.88	2.33	8.60	5.19	4.57	2.63
Protein (µg/mL)	17.56	08.52	36.67	29.11	27.86	18.33
Glutathione (mg/dL)	492.00	502.85	497.60	509.75	496.95	462.35

Bold text: indicates the highest concentration.

RA: Regular Arabica Coffee.

RR: Regular Robusta Coffee.

SDA: Spray-dried Arabica Soluble Coffee.

SDR: Spray-dried Robusta Soluble Coffee.

FDA: Freeze-dried Arabica Soluble Coffee.

FDR: Freeze-dried Robusta Soluble Coffee.

4 DISCUSSION

This study obtained soluble coffee (instant coffee) from freshly/regular ground-roasted coffee beans. Regular ground-roasted coffee (coffee powder), both Robusta and Arabica (medium roasted) were purchased from the same factory (State Coffee Plantation PTPN XII Indonesia). It provided the same coffee origin and processing to minimize confounding factors that might affect the antioxidant activity and the concentration of phytochemicals.

This study primarily focused on comparing freeze-dried and spray-dried soluble coffee. The soluble coffees were prepared by dehydrating regular coffee brews using freeze-drying and spray-drying techniques. Before dehydration, Arabica and Robusta coffee brews were filtered using the same 100 mesh filter to achieve uniform particle size and eliminate particle size as a variable in the comparison between freeze-dried and spray-dried coffee brews. Thus, this study could focus on comparing antioxidant activity and the concentration of phytochemicals between freeze-dried and spray-dried coffee, both Arabica and Robusta coffee. In addition, this study also analyzed the comparison of soluble and regular coffee.

A comparison of regular and soluble coffee in the present study showed that soluble coffee (both Arabica and Robusta coffee) exhibited higher antioxidant activity than regular coffee. This result is in line with a study by Marcucci et al. (2017). The higher antioxidant activity in soluble coffee was aligned with the higher concentration of phytochemicals (polyphenols, protein, caffeine, and glutathione) compared to regular coffee. The explanation might be due to the particle size (Barroso et al., 2024). Soluble coffee consists of smaller particle sizes and can dissolve completely in water. Smaller particle sizes result in a larger surface area in contact with water, allowing for the dissolution of bioactive compounds and a higher concentration of antioxidant compounds.

This study highlights the preservation of antioxidant compounds in freeze-dried soluble coffee (Barroso et al., 2024; Marcucci et al., 2017; Vareltsis et al., 2020) compared to spray-dried soluble coffee. A study by Ghirșan and Miclăuș (2017), however, revealed that spray-dried coffee powder shows higher antioxidant activity in comparison with freeze-dried coffee. They explained that the lower antioxidant activity of freeze-dried coffee was due to the duration of the drying method which was larger for freeze-drying (hours) compared to the spray-drying (second). These contradictory results indicate that the technological process used to produce soluble coffee is an important factor in determining its antioxidant capacity.

Polyphenol levels in freeze-dried soluble coffee, both Arabica and Robusta, were much higher compared to spray-dried and regular coffee. In addition to polyphenols, Robusta soluble coffee also demonstrated a higher level of caffeine and protein. Regarding the concentration of caffeine, spray-dried

Arabica soluble coffee (SDA) demonstrated a lower level of caffeine compared to freeze-dried soluble coffee. Caffeine was likely degraded during spray-drying due to the high temperature.

An interesting result was the level of GSH. Arabica coffee (regular and freeze-dried) showed a high level of GSH. The molecule of GSH is a tripeptide consisting of three amino acids glutamate-cysteine-glycine and is well-known as a master antioxidant (Kozieł et al., 2024). Dietary GSH supplementation enhances antioxidant activity (Liu et al., 2020). Glutathione can scavenge reactive oxygen species (ROS) and protect the sulfhydryl group (-SH) of biomolecules such as proteins. Considering GSH as a peptide molecule, coffee GSH might be produced from the degradation of proteins after being roasted. Further studies were needed to confirm this notion. Studies concerning GSH in coffee are rare.

Protein content in freeze-dried robusta soluble coffee demonstrated the highest levels among the different coffee types tested. In the present study, the high content of protein and polyphenols contributed to the high antioxidant activity. Further studies are needed to clarify the role of coffee protein in antioxidant activity. In addition, protein might act as a source of amino acid antioxidants. Coffee proteins have rarely been studied in the context of their antioxidant activity and health benefits.

Studies on the antioxidant activity of coffee primarily focus on the role of phytochemicals, particularly secondary metabolites like phenolic compounds. Coffee contains a wide range of bioactive compounds (Saud; Salamatullah, 2021), including both secondary and primary metabolites. The antioxidant activity and health benefits of coffee may result from the interactions of various compounds within it. Several factors influence the level of antioxidants in coffee brews, including coffee type and origin, processing methods, brewing techniques, and analytical methods. Furthermore, the health benefits of coffee require further investigation through *ex vivo*, *in vivo*, and clinical trials.

5 CONCLUSION

In conclusion, freeze-dried instant coffee demonstrated more sensitive antioxidant activity, in line with its more abundant polyphenols, caffeine, and protein. Arabica coffee (regular and freeze-dried) contains higher GSH compared to robusta coffee and spray-dried arabica coffee.

6 ACKNOWLEDGMENTS

This study was supported by funding from the Center of Research and Community Development Universitas Jember, contract number 3092/UN25.3.1/LT/2024. We thank the Integrated Analytical Laboratory Faculty of Agriculture Technology and Pharmaceutical Analytical Chemistry

Laboratory Faculty of Pharmacy of Universitas Jember for helping us with the laboratory work.

7 AUTHOR CONTRIBUTIONS

Conceptual Idea: Susilawati, IDA.; Methodology design: Susilawati, IDA.; Muzeka F.; Data collection: Susilawati, IDA.; Muzeka, F.; Data analysis and interpretation: Muzeka, F., Susilawati, IDA.; Writing and editing: Susilawati, IDA.; Muzeka F.

8 REFERENCES

- ALNSOUR, L. et al. Quantification of total phenols and antioxidants in coffee samples of different origins and evaluation of the effect of degree of roasting on their levels. **Molecules**, 27(5):1591, 2022.
- AL-OWAISI, M. et al. GC-MS analysis, determination of total phenolics, flavonoid content, and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. **Asian Pacific Journal of Tropical Biomedicine**, 4(12):964-970, 2014.
- BARROSO, L. A. et al. Impact of the use of cryoprotectants in the production of freeze-dried soluble coffee from cold brew arabica coffee. **Food and Bioprocess Technology**, 17:4754-4767, 2024.
- BELAY, A. et al. Measurement of caffeine in coffee beans with UV/vis spectrometer. **Food Chemistry**, 108(1):310-315, 2008.
- CRIPPA, A. et al. Coffee consumption and mortality from all causes, cardiovascular disease, and cancer: A dose-response meta-analysis. **American Journal of Epidemiology**, 180(8):763-775, 2014.
- DI GIACOMO, C. et al. Natural compounds and glutathione: Beyond mere antioxidants. **Antioxidants**, 12(7):1445, 2023.
- DLUDLA, P. et al. Potential benefits of coffee consumption on improving biomarkers of oxidative stress and inflammation in healthy individuals and those at increased risk of cardiovascular disease. **Molecules**, 28(18):6440, 2023.
- GHIRIŞAN, A.; MICLĂUŞ, V. Comparative study of spray-drying and freeze-drying on the soluble coffee properties. **Studia Universitatis Babes-Bolyai Chemia**, 62(4):309-316, 2017.
- GÓRECKI, M.; HALLMANN, E. The antioxidant content of coffee and its in vitro activity as an effect of its production method and roasting and brewing time. **Antioxidants**, 9(4):308, 2020.
- GROSSO, G. et al. Coffee, caffeine, and health outcomes: An umbrella review. **Annual Review of Nutrition**, 37:131-156, 2017.
- GUNTER, M. J. et al. Coffee drinking and mortality in 10 European Countries: A multinational cohort study. **Annals of Internal Medicine**, 167(4):236-247, 2017.
- KIM, H.; KANG, S. H.; KIM, S. H., Drinking coffee enhances neurocognitive function by reorganizing brain functional connectivity. **Scientific Reports**, 11:14381, 2021.
- KOZIEL, E. et al. Glutathione the “master” antioxidant in the regulation of resistant and susceptible host-plant virus interaction. **Frontiers in Plant Science**, 15:1373801, 2024.
- LIU, J.-D. et al. Dietary glutathione supplementation enhances antioxidant activity and protects against lipopolysaccharide-induced acute hepatopancreatic injury and cell apoptosis in Chinese mitten crab, *Eriocheir sinensis*. **Fish & Shellfish Immunology**, 97:440-454, 2020.
- MARCUCCI, C. T. et al. Antioxidant activity of commercial soluble coffees. **Beverages**, 3(2):27, 2017.
- MARTINI, D. et al. Coffee Consumption and oxidative stress: A review of human intervention studies. **Molecules**, 21(8):979, 2016.
- MOKOROANE, K. T. et al. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts from *Aloisampelos striatula*. **Food Research**, 4(6):2062-2066, 2020.
- POOLE, R. et al. Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. **BMJ**, 359:j5024, 2017.
- PRODIĆ, I. et al. Antioxidant properties of protein-rich plant foods in gastrointestinal digestion-peanuts as our antioxidant friend or foe in allergies. **Antioxidants**, 12(4):886, 2023.
- SAUD, S.; SALAMATULLAH, A. M. Relationship between the chemical composition and the biological functions of coffee. **Molecules**, 26(24):7634, 2021.
- VARELTZIS, P. et al. Production of instant coffee from cold brewed coffee: Process characteristics and optimization. **Food Science and Applied Biotechnology**, 3(1):39-46, 2020.
- YASHIN, A. et al. Antioxidant and antiradical activity of coffee. **Antioxidants**, 2(4):230-245, 2013.