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Analysis of Caffeine Content and other Mineral Components in Different Coffee Germplasms Grown in Pokhara, Nepal

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Abstract
Caffeine present in coffee is the main reason to make it a world-famous drink. It poses health benefits if consumed in limit. Other than caffeine it contains mineral components that poses health benefits if consumed in limit. Nineteen coffee germplasms; exotic and local collections were studied for their caffeine and mineral components in respective two years. Caffeine content of the tested germplasms was ranged from 0.27 to 0.75 in 2016 and 0.32 to 1.02 in 2017. Study of other mineral components like crude protein (CP%), crude ash (CA%), crude fibre (CF%), phosphorus (P), calcium (Ca) and Iron (Fe) showed and an interesting relationship with caffeine content in specific germplasms. The percentile difference in mineral components was positive among the high and low caffeine-producing germplasms in general. High caffeine-containing germplasm was reported with less chemical components and vice versa in the overall analysis. This study supports the value of antioxidant property of coffee drink to protect the body against diseases. Further studies are needed to clarify the effect of the environmental condition and such properties of coffee.
Keywords: antioxidants; coffee germplasm; caffeine; mineral components; health benefits

Introduction
Coffee is a world-famous beverage, with more than 400 billion cups consumed each year. The coffee bean is considered a very important commodity in terms of trading which stands at third

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place among the legally traded products in the world. Global coffee production reached to 10.3 billion kg as of 2018/2019, up from 9.5 billion kg in 2017/2018 (Shahbandeh, 2020).

The active ingredient that makes coffee valuable is caffeine. Caffeine is an alkaloid, which is a class of naturally occurring compounds containing nitrogen and having the properties of organic amine bases. Coffee becomes addictive because of the chemicals in the coffee and caffeine is the main one. Caffeine, the most studied and consumed stimulant in the world, is well-known for its beneficial and/or adverse effects on human health (Bunker and McWilliams, 1979; Nawrot et al., 2003; Meredith et al., 2013; Wolde, 2014; Gaeini et al., 2019). It stimulates by blocking neuroreceptors for sleep chemical adenosine. When the sleep chemical is blocked by caffeine, it keeps the coffee drinker awake. Caffeine withdrawal can cause headaches and irritability thus many people prefer not to give up their coffee. Over the last decade, it has been concluded that consumption of caffeine is not harmful if consumed in a limit of 400 mg per day (equivalent to around 5 cups of coffee) for most healthy adults (Smith and Fuller, 2016; Ding et al., 2014; Nawrot et al., 2003).

Caffeine is an antioxidant which is linked to several potential health benefits like negative or some protective effects on cancer (Yu et al., 2011; Wang et al, 2016). It helps in reducing the risks of several chronic diseases (Kurozawa et al., 2005; Zhang et al, 2015; Park et al., 2016), as well as in improving immune function but also risks of developing coronary artery disease, osteoporosis, gastritis, iron deficiency anemia, and stillbirths (IFIC, 2003). It is also reported that caffeine can interfere with the nutrient absorption of essential minerals, including calcium, iron, magnesium, and B vitamins (Escott-Stump, 2008). The beneficial health effects of coffee are usually attributed to its high antioxidant activity (Janda et al., 2020; Belitz et al., 2009).

Two of the coffee species; *Coffea arabica* and *Coffea canephora* (or *Coffea robusta*) are popular throughout the world. In Nepal, mostly *Coffea arabica* is common. The coffee grown in Nepal is considered of very high quality because it is mostly grown in hills and mountains in the country. Provided that the roasting and packaging are done right, the taste of Nepal-grown *arabica* coffee is quite ideal. This study was done to assess the caffeine content in the coffee grown in Nepal and to figure out whether the caffeine and mineral components are related with each other or not.

Materials and Methods

The study site and coffee germplasms

The coffee orchard in this study is located at the Horticulture Research Station, Malepatan, Kaski, Nepal along the latitude 28.2179' and longitude 83.9730'. The climate of the site is humid and sub-tropical. The average annual rainfall is 4000 mm. Coffee germplasms are grown in the litchi-coffee shade system and planted at the ratio of 1:4. The layout design is randomized complete block design (RCBD) with four replications.

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Altogether 19 coffee germplasms namely Yellow Catura, Chhetradeep, Syangja Special, Arghakhanchi Local, Catui Amarillo, Selection-10, Tekisic, Mundo Novo, Bourbon Amarillo, Bourbon Vermelo, Caturra Amarillo, Caturra Vermelo, Pacamara, Catui Vermelo, Kaski Local, Indonesis, Hawai Kuna, Pacas and Catimor were selected. Among the 19 germplasms Chhetradeep, Syangja Special, Arghakhachi Local and Kaski Local were local collections of respective locations and rest were exotic. All germplasms belong to *Coffea arabica*. The composite samples for each variety used in this study.

Sample preparation for chemical analysis

Fresh coffee cherry samples were collected by hand picking at the end of January in the years 2016 and 2017. The collected fresh cherries were subjected to pulping within 24 hours, which were further subjected to fermentation by mixing equal amount of water for 72 hours and washed thrice with clean water to remove the mucilaginous substances of coffee parchment. All parchments were dried under coffee drier for about 10-12 days until a constant weight was achieved. Green beans were obtained from de-hulled parchment with a moisture percentage of 10-11%. Green beans were subjected to roasting in the roasting machine for 15 minutes at the temperature of 150° C (initial) to 200° C (end point temperature). The roasted beans were grounded in grinding machine to obtain filter-grade coffee.

Chemical analysis

Crude protein (CP) in the grinded coffee samples was determined by Kjeldahl method, fat by Ether Extractive method and ash content was determined by using a muffle furnace. The ash obtained by dry ashing, then 25 ml of 10% HCl was added and then boiled for 5 minutes. The solution was filtered, and the filtrate was collected into the 100 ml volumetric flask and made up to volume with distilled water. The ash solution hence made is used for the determination of minerals. Analysis was done as per the official method of Analysis of the Association of Official Analytical Chemists (AOAC, 2005). Phosphorous and iron content was determined in Spectrophotometer as described by AOAC (2005). Caffeine was extracted by using dichloromethane and analyzed as the method described by Gerald *et al.,* 2014. Total polyphenol content (TPC) was determined using a modified Folin-Ciocalteu Colorimetric method (Huang *et al.,* 2012) and the results were expressed as milligrams of Gallic acid equivalents per 100 g dry weight (g GAE100 g/ DW).

Statistical analysis

The raw data were entered in MS-Excel, averaged and presented in bar diagram. Percentile analysis was done in Excel using PERCENTILE.INC function, which calculates the k-th percentile of a range data. Chemical properties of coffee beans in high and low caffeine containing germplasms were arranged in table using MS-Excel.

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Results

Chemical properties of coffee beans among the germplasms

The chemical properties recorded in this study were varied within germplasms and years (Table 1). Among the tested germplasms, the level of caffeine ranged from 0.33 to 0.75 and 0.32 to 1.02 in the year 2016 and 2017, respectively. In the year 2016, the lowest caffeine was observed in 'Indonesia' whereas highest was in 'Kaski Local'. In 2007, the same germplasm 'Indonesia' was recorded with the lowest caffeine, whereas highest caffeine was recorded in 'Pacas'. Crude protein (CP) was ranged from 11.89% to 19.05% and 11.53% to 17.60% in 2016 and 2017, respectively. Germplasm 'Indonesia' had the highest CP in both the years. Germplasms 'Pacas' and 'Pacamara' recorded with lowest CP in 2016 and 2017, respectively. Crude ash (CA) was 4.00% to 4.97% in 2016 and 3.69% to 4.86% in 2017. Similarly, crude fiber (CF) ranged from 8.42% to 22.83% and 11.18% to 18.42% in 2016 and 2017, respectively. The highest CF was recorded in 'Kaski Local' in both years. Phosphorus (P) content ranged from 6.21 ppm to 20.02 ppm in 2016 and 9.9 ppm to 18.62 ppm in 2017. In both years, the highest P was found in 'Yellow Caturra' and the lowest in 'Selection 10'. The level of calcium (Ca) ranged from 257 ppm to 445 ppm in 2016 and 318.42 ppm to 468.76 ppm in 2017. Likewise, the iron (Fe) content was highest (11.93 ppm and 9.36 ppm) in 'Pacas' and lowest (1.03 ppm and 1.19 ppm) in 'Pacamara' in the year 2016 and 2017, respectively.

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Table: 1

Chemical property of different coffee beans among different germplasms

S.N	Germplasm	Caffeine (%DB)		CP (%)		CA (%)		CF (%)		P (ppm)		Ca (ppm)		Fe (ppm)	
		2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
1	Yellow Caturra	0.52	0.64	15.9	16.2	4.52	4.4	10.04	12.2	20.02	18.62	370	392.34	4.37	6.58
2	Chhetradeep	0.64	0.92	17.54	13.62	4.2	3.81	11.21	16.54	9.9	12.16	320	364.63	2.83	4.76
3	Syangja Special Arghakhachi	0.69	0.74	14.01	14.16	4.17	4.24	10.33	13.28	15.88	14.64	314	358.38	2.08	4.18
4	Local	0.63	0.68	13.61	14.02	4.46	4.2	10.22	11.18	11.93	13.2	347	365.8	4.75	5.88
5	Catui Amarillo	0.59	0.61	12.1	13.8	4.2	4.86	12.9	14.92	11.85	12.68	328	334.65	4.53	5.69
6	Selection (10)	0.65	0.58	14.26	15.4	4.97	4.76	10.36	12.8	6.21	9.9	257	324.28	2.22	3.35
7	Tekisic	0.4	0.62	17.51	14.59	4.1	3.78	8.42	17.72	10.63	12.64	444	412.26	3.6	4.12
8	Mundo Novo Bourbon	0.38	0.46	15.54	16.24	4.37	4.08	10.87	16.68	14.77	13.36	445	418.16	4.22	3.78
9	Amarillo Bourbon	0.44	0.42	16.91	16.02	4	3.96	12.61	14.74	7.11	10.12	338	382.78	1.94	2.63
10	Vermelo Caturra	0.58	0.48	16.15	15.78	4.95	4.38	15.88	16.62	12.46	14.44	337	370.18	3.82	4.18
11	Amarillo Caturra	0.38	0.4	16.38	15.96	4.9	4.48	16.4	15.96	9.52	10.71	250	316.24	4.94	5.68
12	Vermelo	0.33	0.38	15.17	13.98	4.24	4.12	13.8	14.82	17.54	16.22	342	383.86	3.67	3.38
13	Pacamara	0.74	0.97	16.64	11.53	4.76	3.69	16.07	17.2	9.3	10.84	363	406.61	1.03	1.19
14	Catui Vermelo	0.64	0.62	14.63	15.2	4.1	4.32	14.29	15.49	9.32	10.92	411	434.56	4.17	3.47
15	Kaski Local	0.75	0.72	16.07	15.88	4.03	4.28	22.83	18.42	9.95	10.78	276	344.81	10.7	8.25
16	Indonesia	0.27	0.32	19.05	17.6	4.4	4.2	20.21	18.2	9.45	10.42	271	335.67	6.08	8.64
17	Hawai Kuna	0.43	0.56	18.19	17.4	4	3.98	18.53	17.42	11.24	12.26	286	318.42	3.07	5.86
18	Pacas	0.53	1.02	11.89	13.42	4.64	3.84	21.27	17.12	14.96	15.84	316	329.91	11.93	9.36
19	Catimor	0.42	0.52	15.28	16.46	4.8	4.24	8.79	14.68	9.46	12.32	423	468.76	2.56	3.75

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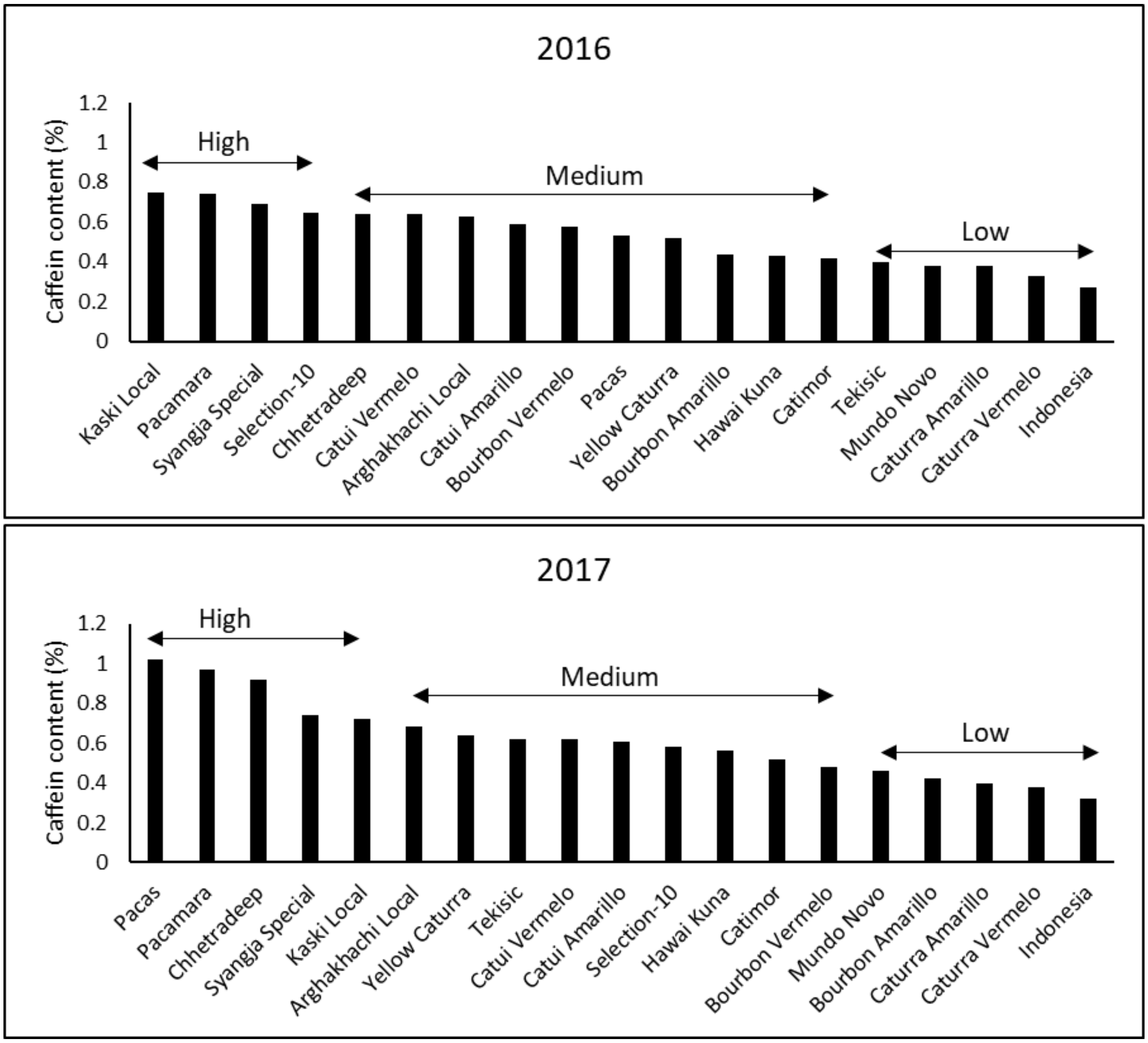
Figure: 1
Caffeine content in different coffee germplasms in 2016 and 2017. Germplasm with high (>75 percentile), medium (75-25 percentile), and low (<25 percentile) caffeine contents in each year are shown by arrows



Caffeine content among the germplasms

Figure 1 shows the ranking of coffee germplams based on caffeine content in the years 2016 and 2017. The germplasms Kaski Local, Pacamara and Syangja Special were ranked in high caffeine content (>75 percentile) in both the years. Likewise, Mundo Novo, Caturra Amarillo, Caturra Vermello and Indonesia recorded a low level of caffeine (<25 percentile) in both the years. Almost half of the tested germplasm produced a medium level of caffeine (25-75 percentile).

Caffeine content in relation to other mineral contents

To see the relation of caffeine contents with other chemical properties and mineral contents in coffee, the values of the CP, CA, CF, P, Ca, and Fe were analyzed in high- and low- caffein containing genotypes (Table 2). In high caffeine-containing genotypes; CP, P, and Fe contents

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were clearly higher than those in the low caffeine containing genotypes, suggesting that the caffeine content is reduced under high nitrogen, phosphorus, and iron fertilizations.

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Table: 2

Chemical properties in coffee beans in high and low caffein containing germplasms

Caffeine level in percentile	CP (%)		CA (%)		CF (%)		P (ppm)		Ca (ppm)		Fe (ppm)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
High (>75)	15.57±1.38	13.86±2.19	4.32±0.39	4.07±0.33	16.41±6.26	16.30±2.69	11.71±3.63	12.09±2.21	317.84±43.25	369.93±32.48	4.60±5.31	4.54±3.54
Low (<25)	16.54±1.75	15.95±1.49	4.48±0.29	4.22±0.18	15.32±3.97	16.42±1.42	12.82±4.01	12.68±2.71	327.22±88.19	363.48±46.23	4.73±1.04	5.37±2.40
Difference (%)	6.18	15.07	3.65	3.69	-6.64	0.71	9.48	4.89	2.95	-1.74	2.70	18.28

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Discussion

Present study shows some variation in caffeine content among the coffee germplasms grown in Nepal. Previous reports indicate that caffeine content in commercial Arabica coffee varies between 1.0 to 1.2 % (Paulo and Silvarolla, 2010). Caffein content in the coffee germplasms in this study was below this range except for Pacas (1.02%) in the year 2017. Most of the germplasms had less caffeine (≤ 0.6%). The only types with less caffeine (0.5-0.6 %) are those such as Mokka and Laurina that carry the laurina genetic factor (Clifford, 1985). Reports showed that the coffee bean (Mozzafera and Silvarolla, 2010) and roast style (Krol et al., 2020) affect the caffeine content in coffee. Caffeine content decreased with intensified roasting (Krol et al., 2020; Hecimovic et al., 2011).

Moreover, this variability can be attributed to variation in the soil, altitude and climate of the coffee growing area (Hameed et al., 2020; Hagos et al., 2018; Gebrekidan et al., 2019). Studies showed the negative correlation between the caffeine contents and the coffee plants grown altitudes (Hagos et al., 2018; and Sherge et al., 2016; Tolessa et al., 2016). Soil and climate also influence other coffees characteristics like chemical content, flavor or aroma (Heaney, 2002; Ashihara et al., 2008). Nepalese coffee is considered specialty coffee for its distinct flavor aroma and body as it is grown in high altitude under suitable climate, topography, soil, relative humidity, temperature and most importantly in organic culture (Subedi, 2011; Karki and Regmi, 2016). Coffee beans produced in organic culture reported with higher level of total phenolic content than in conventional one (Krol et al., 2020). Mozzafera and Silvarolla (2010) reported around 1.16 % of caffeine in varieties Bourbon Vermelo and Catui Vermelo. In current study, the same two varieties were observed with around 0.5% and 0.6 % of caffeine respectively.

Some of the genotypes showed a similar trend in high, medium and low levels of caffeine production both the year. Some exceptional case for example; Pacas in 2016, it was 0.53% whereas in 2017 it was recorded almost double i.e. 1.02%. A slight variation in caffeine content of specific germplasm in two respective years might be due to the 10% of cross pollination reported for coffee (Mazzafera and Silvarolla, 2010) or genetic control of caffeine biosynthesis and catabolism (Ashihara et al., 2008).

An interesting relationship between caffeine and other components was observed in this study. The mineral components were positively related to caffein content (except for CF% in 2016 and Ca in 2017). Mazzafera (1999) has reported that caffeine content was decreased from 2.2 to 1.75% in the coffee leaves when P, N, Mg, and Ca were omitted in the soil. In this study, the germplasms with less caffeine content showed a higher accumulation of mineral components, which is a good news for the coffee lovers.

Conclusion

The caffeine content in coffee plays an important role in its popularity. Besides caffeine, coffee is a good source of other mineral components which has many health benefits. There was a big difference in caffein content among the tested germplasms in this study. Interestingly, caffeine content was related to other mineral content. It can be concluded that a normal dose of coffee intake may provide essential nutrients other than the antioxidants. Since caffeine and mineral components varied within genotypes and growing conditions; there should be further study on the role of these factors on its quality.

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Authors' Contributions: The authors conducted all research activities i.e., concept, data collecting, drafting and final review of manuscript.

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