Journal of Food Processing & Technology

Aroyeun and Jayeola, J Food Process Technol 2016, 7:4
DOI: 10.4172/2157-7110.1000579

Research Article Open Access

Effects of Green Tea Extracts on the Caffeine, Tannin, Total Polyphenolic Contents and Organoleptic Properties of Milk Chocolate

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Abstract

Chocolate and green tea contains antioxidants that may be used as health promoting foods. A new product incorporating green tea in Chocolate and termed green tea chocolate was developed in this project. Green tea was produced using the Chinese method viz: Plucking, Fixing, Rolling and Drying. The green tea produced was milled into powder and mixed at different ratios: 10:90, (GTCE) 20:80 (GTCD), 30:70 (GTCC), 40:60 (GTCB) and 50:50 (GTCA) (w/w) of the green tea powder to the Chocolate. Conventional milk chocolate (GTCF) without green tea served as the control for the production of chocolate followed by the standard method. Proximate Analysis, Total Phenol and antioxidant properties were determined using standard methods; Sensory Analysis was done by panel of tasters who measured the Taste, Odour, flavour and general acceptability.

The average protein of the control chocolate was 8.05 which varied between 7, 24% (10% supplementation) and 8.39% (50% supplementation). The % crude fibre for the control chocolate was 1.17% and for other supplemented levels, it varied from 0.93% for 10% green tea addition to 1,23%. All the crude fibre increased significantly ($p < 0.05$) on increasing the green tea in the chocolate. It was found that at 10% inclusion of green tea, there were increases of 9.7%, 9.6%, 4.4% and 3.2% in crude fibre as compared to the control. This may be due to higher contents of dietary fibre in the green tea. The percentage total ash of the control was 2.43% and increased significantly and consistently from 2.28 at 10% level of inclusion to 2.55 at the 50% green tea supplementation.

The chocolate containing the smallest quantity of green tea powder seemed to have the lowest amount of crude fat and rose steadily with increase in the green tea supplementation. Although at these levels the effect of the crude fat on the chocolate was not significant while the significance was observed only at 50% green tea inclusion. The control chocolate had 162.39 mg/100 g gallic acid equivalent and it increased significantly ($p < 0.05$) with rise in green tea powder in the recipe. This study also established that at the organoleptic threshold of 10%, green tea supplementation, there was no significant difference in the polyphenol content of the chocolate. It became significant at higher level of inclusion, i.e. 40-50% at which the taste of the chocolate and the overall acceptability and colour and sweetness became impaired. The caffeine content increased with increase in green tea powder in the chocolate. Green tea inclusion at 20%, 30%, 40% and 50% were showing significantly better chelating properties than the control samples and the 10% samples. The iron chelating ability increased with high contents of green tea and in the order 50% > 40% > 30% > 20% > control > 10%. The L* value reduced significantly from 10% to the 50% green tea powder.

In conclusion, replacement of cocoa nibs with green tea powders up to 20-50% impaired the taste of the chocolate.

Keywords: Chocolate; Tea powders; Caffeine

Introduction

Chocolate and green tea contains antioxidants that may be used as health promoting agents. Tea and tea products mainly contain tea polyphenols, which are natural antioxidants and have been demonstrated to show antioxidative, anti-carcinogenic and anti-microbial properties by many researchers [1,2]. These health benefits of teas, in particular green tea, are gaining increased attention in recent years. Green tea contains the most abundant tea polyphenols, namely tea catechins. The major nutraceutical Compounds in green teas are tea catechins, which are flavanols. Flavanols are a class of flavonoids which are polyphenols. Green tea is rich in flavanols (300-400 mg/g) which are of interest to human health [3]. Tea catechins have the most effective antioxidant activity compared to other Tea polyphenols. The major green tea catechins are (–)-epigallocatechin gallate (EGCG), (–) -epicatechin gallate (ECG), (–)-epigallocatechin (EGC) and (–) -epicatechin (EC). These epicatechins can change to their epimers that are non epicatechins, i.e. (–) -gallocatechin gallate (GCG), (–) -catechin gallate (CG), (–)-gallocatechin (GC) and (±)-catechin C) Figure 1. EGCG is the most abundant and active catechin and it is usually used as a quality indicator [4-6]. In addition, green tea contains other polyphenols such as gallic acid, quercetin, kaempferol, myricetin and their glycosides, but at lower concentration than EGCG [3-7].

Tea catechins are an efficient free radical scavenger due to their one electron reduction potential. A lower reduction potential has a tendency to lose electron or hydrogen [8]. The rate of reaction with free radicals and the stability of the resulting antioxidant radicals contribute to the reactivity of antioxidant. Guo [9] reported the scavenging ability of tea catechins on superoxide anions (O^{2-}), singlet oxygen, the free radicals generated from 2,2P-azobis (2-amidinopropane) hydrochloride (AAPH) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. They suggested that the scavenging ability of EGCG and GCG was higher than that of EGC, GC, EC and C due to their gallate group.

They are nowadays utilized in a wide range of applications, such as food, beverage, cosmetics toiletries etc. [10]. The consumption of green tea in the form of hot beverages is common in China and Japan

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Received March 22, 2016; **Accepted** April 15, 2016; **Published** April 21, 2016

Citation: Aroyeun SO, Jayeola CO (2016) Effects of Green Tea Extracts on the Caffeine, Tannin, Total Polyphenolic Contents and Organoleptic Properties of Milk Chocolate. J Food Process Technol 7: 579. doi:10.4172/2157-7110.1000579

and the incorporation of it either in the form of powder or in the form of extracts in foods are available in the literature. Somboonvecharkarn [11] reported the effect of green tea extract in soy bread and observed that green tea fortified soy bread was similar to the regular soy bread in some physical properties. In the food industry, both chocolate and green tea is popular choices for health benefits and also their reasonable price compared to other dietary supplements such as Ginseng and Ginko leaves. Chocolate and green tea has been associated with antioxidant properties which are also linked to prevention of cancer, cardiovascular disease and as antiobesic effects [12]. Catechin and polyphenol compounds in green tea are excellent antioxidants that are enhanced by the presence of metals [13]. Studies have shown that 2 grams of green tea have equivalent antioxidant activity of 109-147 mg epigallocatechin gallate (EGCG), 14 muM for Catechin and 22 muM for vitamin C [14]. Although the popularity of green tea and chocolate is growing in the United States, there are no records in the literature where the two were combined. The objectives of this work, however, are to create a novel food product incorporating the combination of chocolate and green tea and determine the chemical and organoleptic qualities of the newly developed product.

Materials and Methods

Fresh tea leaves were harvested from the Mambilla Highland; Taraba State, Nigeria Green Tea was processed using the Chinese method viz: Plucking, withering, Fixing, Rolling and Drying. Ingredients of Chocolate were mixed together during Conching and the weight was taken. The incorporation of green tea was based on different combination such as 10:90, 20:80, 30:70, 40:60, 50:50 (w/w) of the green tea powder and chocolate, the flow chart of chocolate processing was shown below in Figure 1 and the Recipe for the production of chocolate was shown in Table 1.

Proximate Analysis

Moisture content

The moisture content of chocolate was determined by drying the samples in an oven at 105°C until a constant weight was obtained (AOAC).

Crude protein

Crude protein content was calculated by converting the nitrogen content, determined by Kjedahl's method (6.25 × N)

Crude fat

This was determined by the method described by the AOAC, using the Soxhlet methods

Total ash

Content was determined by dry ashing in a furnace at 525°C for 24 h.

Caffeine determination

This was done in accordance with Yao [15]. Tannin Analysis was carried out using the method of Somboonvecharkarn [11].

Total Phenol Determination

Extraction procedure

Known weight of the green tea chocolate was ground and transferred into a test tube and mixed with 10 ml of 80% methanol. Suspension was vortexed and centrifuged for 10minutes. The mixture was sonicated for 5 minutes, then shaken at 120 rpm at 70°C for 2 hours. It was then centrifuged for 10minutes. Supernatants were collected and filtered.

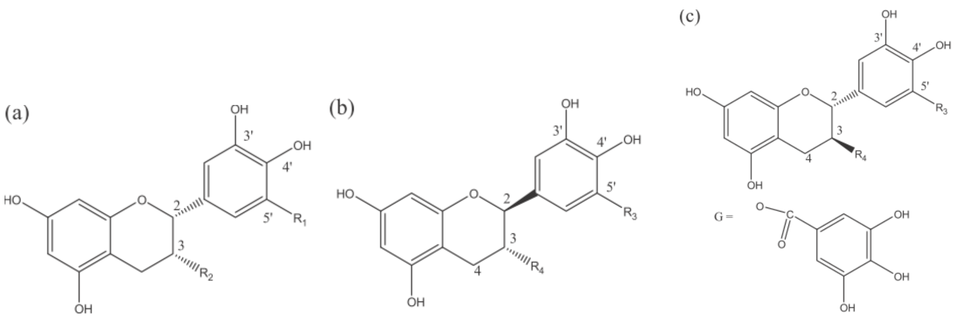

Figure 1: General chemical structures of green tea catechins: (a) epi-catechins; (b) non-epicatechins and (c) (+)-catechin.

Samples %	Tea	Nibs	Milk	Sugar	Cocoa Butter	Lecithin
10%	2.8	25.96	20.77	47.3	2.5	0.51
	11.25g	101.25	81.00	184.5	10	2.0
20%	5.77%	23.08	20.77	47.30	2.5	0.51
	22.5g	90	81.0	184.5	10	2.0
30%	8.65%	20.19	20.77	47.3	2.5	0.51
	33.75g	78.75	81.0	184.5	10	2.0
40%	11.56%	17.31	20.77	47.30	2.5	0.51
	45.10g	67.50	81.0	184.5	10	2.0
50%	14.42%	14.42	20.77	47.3	2.5	0.51
	56.25g	56.25	81.0	184.5	10	2.0

Table 1: Recipe used in green tea production in this study.

Folin-Dennis Ciocalteau procedure

Total phenolic content was estimated by the Folin-Ciocalteau colorimetric method based on the procedure of Singleton and Rossi, 1965 using Gallic acid as a standard phenolic compound. 100 µl of the filtered extracts were mixed with 400 µl of 80% methanol and 2.5 ml of 0.2N Folin Ciocalteau phenol reagent. After 5 minutes, 2 ml of 7.5% sodium carbonate was added. The absorbance of the resulting blue-coloured solution from yellow solution was measured at 765 nm spectrophotometrically after 30 minutes in the dark at room temperature. Quantitative measurements were performed based on a standard curve of Gallic acid [16]. The total phenolic content was expressed as Gallic acid equivalents (GAE) in mg/g dry material

Chelating effect on ferrous ions

The ferrous ion chelating activity of the green tea chocolates was assessed as described by Tawaha K [17]

Colour analysis

For each sample, colour was determined with the portable Minolta Chromameter CR (Minolta, Osaka, Japan). The Lab values follow the Hunter Lab color scale

Sensory analysis

The sensory Analysis was carried out in accordance with standard methods (Figure 2).

Statistical analysis

Samples were analyzed in triplicates. Means were separated using ANOVA.

Results and Discussion

From the univariate statistical analyses, all chemical properties measured varied significantly ($p < 0.05$) among the chocolate samples.

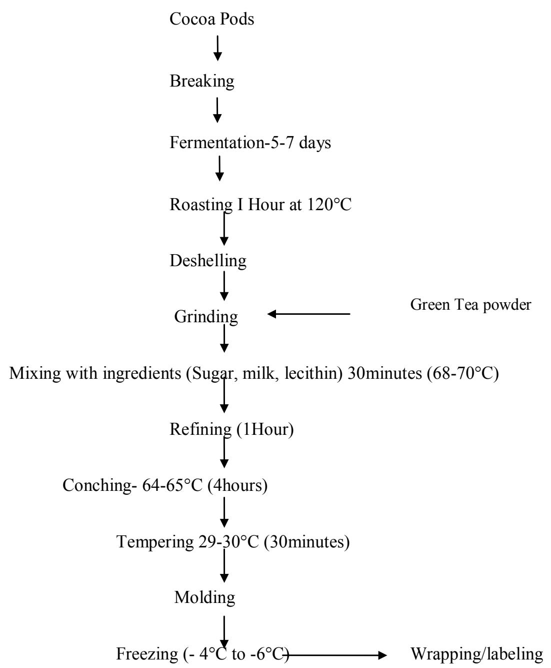

Figure 2: Flow chart for green tea chocolate production.

Some of the results were shown in Tables 1–4 while the summary has been displayed in the boxplots (Figure 1). This directly reflected in the smaller thickness of the boxes and deviation from the means (depicted by the protruding bars) of the former compared to the latter. According to Table 1, the proximate chemical composition of the chocolates such as the crude protein, crude fibre, total ash, moisture content, sucrose and crude fat differed significantly ($p < 0.0\text{-}5$) with increase in the levels of the green tea added. The average protein of the control chocolate was 8.05 whereas that of chocolate supplemented with green tea varied accordingly. The protein values varied from 7.24 for 10%, 7.65% for 20%, 7.93% for 30%, 8.27% for 40% and 8.39% for 50% green tea supplementation respectively.

The % crude fiber for the control chocolate was 1.17% and for other supplemented levels, it varied from 0.93% for 10% green tea addition, 1.03 for 20% addition, 1.14 (30%) 1.18 (40%) and 1.23 (50:50). The percentage total ash of the control was 2.43% and increased significantly and consistently from 2.28 at 10% level of inclusion to 2.55 at the 50% green tea supplementation. The sucrose level of the control was 48.3% and the sucrose decreased significantly and steadily from 10% green tea inclusion to the 50% level. This is an expected trend; however, as green tea is bitter and the reduction in sucrose level is an indication of the significant impact of the bitterness on the reduction in sweetness of the chocolate. As for the % crude fat, the control chocolate had the highest % amount while the samples in which green tea has been supplemented increased steadily and significantly with increased amount of green tea powder.

The chocolate containing the least inclusion seemed to have the lowest amount of crude fat and rose steadily with increase in the green tea supplementation. Although at these levels the effect on the crude fat on the chocolate was not significant while the significance was observed only at 50% green tea inclusion. This might be due to the fat content in green tea which reflected in the crude fat of the chocolate. All the crude fibre increased significantly ($p < 0.05$) on increasing the green tea in the chocolate. It was found that at 10% inclusion of green tea, there were increases of 9.7%, 9.6%, 4.4% and 3.2% in crude fibre as compared to the control. This may be due to higher contents of dietary fibre in the green tea. This result is in agreement with the work of Guo [9]

Polyphenols and tannin

The control chocolate had 162.39 mg/100 g gallic acid equivalent and it increased significantly ($p < 0.05$) with rise in green tea powder in the recipe Table 2. The maximum increase of 28.4% in polyphenol over the control chocolate was found in 50% green tea inclusion while an increase of 12.45% of the polyphenol was found in chocolate with green tea supplemented at 40%. There was no significant effect of green tea addition on the polyphenol profile of the chocolate at 10%, 20% and 30% respectively. This in effect confirmed that chocolate itself contains polyphenols which made the addition of green tea powder

Samples	% Crude Protein	% Crude Fibre	% Total Ash	% Moisture Content	% Sucrose	% Crude Fat
10%	7.24	0.93	2.28	2.15	49.18	31.28
20%	7.65	1.03	2.41	2.06	48.25	33.42
30%	7.93	1.14	2.37	2.02	46.13	35.84
40%	8.27	1.19	2.49	1.24	45.74	37.52
50%	8.39	1.23	2.55	1.15	43.25	39.34
Control	8.05	1.17	2.43	1.41	48.36	38.12

Table 2: Proximate analysis of chocolate formulated with different levels of green tea powder.

to be insignificant on the polyphenol of the chocolate. However, no report is found in the literature confirming which of the two samples i.e. green tea or chocolate has the higher polyphenols content. It was only established that both of them are polyphenolic products.

This study also established that at the organoleptic threshold of 10%, green tea supplement, there was no significant difference in the polyphenol content of the chocolate. It became significant at higher level of inclusion, i.e. 40-50% at which the taste of the chocolate and the overall acceptability and colour and sweetness became impaired. The polyphenol contents of chocolate at these two levels had earlier been reported by Mexis [18] who reported that the beneficial effect of tea derived primarily from ingredients such as antioxidant substances (polyphenols).This means that the possibility of 40% and 50% Green tea powder addition in chocolate recipe can cause an increase in the beneficial effect of chocolate consumption, because, there is a synergy of polyphenol between green tea and chocolate [19-21]. The tannin increased as green tea powder increased but it was not significant until at 40-50% when it became significant. In our study, it was also observed that % tannin of all the examined green tea chocolates and the control was lower (Tables 3-5).

Caffeine

The caffeine content in Table 2 increased with increase in green tea powder in the chocolate. Up to 30% inclusion, the green tea did not have any significant effect on the chocolate ($p < 0.05$). Caffeine is a major component of tea, coffee, kola and cocoa [14] although it is a stimulant; excess of it can cause impairment of the mechanical properties of growing bone in early life. According to Table 2, the 20.8% increase in caffeine levels of the control chocolate over the 10% supplementation is desirable for low caffeine consumers. Since the sensory threshold of the chocolate remains at 10%, the reduction of the caffeine level is a welcome development. This has also been supported by Chand [19], who reported that it is practically impossible to avoid caffeine as it is present in various foods and beverages and over the counter medications [14]. On balance, it is better to reduce the daily intake of caffeine.

Samples	Iron Chelating agent mg/100 g	Tannin (%)	Caffeine (%)	Total Polyphenol mgGAE/100 g
10%	1.84	0.0021	0.053	118.46
20%	1.76	0.0029	0.062	124.84
30%	1.69	0.0035	0.069	151.65
40%	1.73	0.0043	0.074	185.49
50%	1.65	0.0051	0.081	226.68
Control	1.79	0.0039	0.067	162.39

Table 3: Polyphenols profiles of green tea chocolate.

Samples	L*	A	b
10%	57.77	2.68	20.22
20%	52.4	2.76	18.64
30%	51	2.84	18.22
40%	48.90	2.88	17.4
50%	42.32	3.42	16.35
Control	58.54	2.66	22.54

Table 4: Colour analysis of green tea chocolate.

Chocolates	Colour	Taste	Smoothness	Sweetness	Overall Acceptability
GTC_a	5.21^c	4.10^c	4.23^d	4.40^d	4.20^d
GTC_b	5.23^c	5.46^b	4.62^d	4.62^d	4.81^d
GTC_c	5.34^c	5.41^b	5.11^c	5.12^c	5.63^c
GTC_d	6.21^b	5.42^b	5.44^b	6.32^b	6.11^b
GTC_e	7.62^{ab}	7.45^a	7.62^a	7.45^{ab}	7.52^{ab}
GTC_f	8.42^a	7.68^a	8.26^a	8.22^a	8.58^a

GTC_a- 50%; Green tea: Cocoa (w/w); GTC_b- 40%; GTC_c- 30%; GTC_d - 20%; GTC_e- 10%; GTC_f- 0%

a, b, c and d : Means along the same vertical columns with different alphabets are significantly different at $p < 0.05$ than those reported in the literature [13]. Decreased tannin concentrations are significantly useful for iron absorption and improved digestion [11].

Table 5: Sensory evaluations of green tea chocolate.

Iron chelators

The ability to chelate transition metals can be considered as an important antioxidant mode of action. In fact, the chelation and deactivation of transition metals prevent these species from participating in hydroperoxidation and decomposition reaction [15]. Green tea inclusion at 20%, 30%, 40% and 50% were showing significantly better chelating properties than the control samples and the 10% samples at 1.76, 1.69, 1.67, 1.65 mg/100mg respectively (Table 2). Since ferrous ions were the most effective prooxidants and are commonly found in vegetables, the high ferrous ions chelating abilities of the 20%, 30%, 40% and 50% of green tea in chocolate would be beneficial. The iron chelating ability as found in this study increased with high contents of green tea and in the order 50% > 40% > 30% > 20% > control > 10%. In fact, the chelating effects of green tea on the chocolate in the listed order were stronger when compared to vitamin C, BHT, and BHA [15]. These data revealed that our chocolate products with green tea demonstrate an interesting capacity for iron binding. In fact, numerous other studies indicated that plant extracts enriched in phenolic compounds are capable of complexing with and stabilizing Transition metal ions rendering them unable to participate in metal-catalyzed initiation and hydroperoxide decomposition reactions [16]

Colour

According to Table 3, Changes in colour parameters L* of chocolate with green tea is a function of aesthetic value. The L* value reduced significantly from 10% to the 50% green tea powder inclusion in the order Control > 10% > 20% > 30% > 40% > 50% representing 58.54, 57.77%, 52.4%, 51.0% 48.9% and 42.32% in values. Different trend was observed in a* value and the b* values without any significant difference. Lower L* value indicated increasing darkness and lower b* value suggested decreasing yellowness. This result was in agreement with McKay [1] who reported that the addition of green tea extract to soy bread increased the darkness of the crumbs and the bread faded from bright yellow to medium brown. This result is not in agreement with Wang [6] who reported an increase in L* values of commercially packaged dark chocolate. The difference in our study and Wang was directly related to the formation of large white spots on the surface of the chocolate known as fat bloom which is not present in our samples.

Sensory of evaluations

The effect of green tea supplementation on the sensory characteristics of chocolate is presented in Table 4. With increase in the levels of green tea in the formulation, the sensory scores for colour, taste, sweetness, flavor and overall acceptability of the chocolate decreased sharply. Replacement of cocoa nibs with green tea powders up to 20-50% impaired the taste of the chocolate. Control samples had the highest scores of 7.68 in taste, which decreased significantly from 7.45 to 4.10 due to the bitter taste of the green tea. The colour of the control samples was scored highest when compared to other chocolate samples where green tea powder was incorporated. The dullness of the chocolate increased with addition of green tea powder in the chocolate mix.

The texture of the chocolate in terms of smoothness decreased according to the green tea percentage inclusion meaning that the higher the green tea powder the lower is the smoothness or the higher is the roughness. The control samples had maximum overall acceptability whereas chocolate containing 40 to 50% were found to be unacceptable to the panelists. The overall acceptability score for control was 8.58 on a 9 point hedonic scale. Chocolates made from blends containing 10% level of green tea powder did not differ significantly from the control sample in Taste, odour, flavor and overall acceptability ($p < 0.05$) (Table 5).

Similar observation with supplementation of soy flour [20], bajol grain flour and wheat flour have also been reported. McKay and Somboonvecharkarn [1,11] also reported similar work on the effect of green tea extract in soy bread physical properties and total phenolic content. For the overall acceptability, ratings, it was concluded that green tea powder could be incorporated into chocolate to 10% without necessarily affecting their sensory quality

Conclusion

In conclusion, replacement of cocoa nibs with green tea powders up to 20-50% impaired the taste and the colour of the chocolate.

Acknowledgement

Authors wish to acknowledge the executive Director/Chief Executive of the Cocoa Research Institute of Nigeria professor, M.O, Akoroda for permission to publish this paper. Mr. Obatoye and Mrs. Ogunlusi of the chocolate laboratory are also appreciated for their efforts to get the chocolate produced despite all odds.

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