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OPTIMIZATION AND EVALUATION OF BIOACTIVE CHARACTERSTICS OF COMMERCIAL *RUMEX ABYSSINCUS* (MEKMEKO) TEA in Ethiopia

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Abstract

The physicochemical parameters, mineral composition, and nutraceutical properties of commercial "mekmeko" teas powder were analyzed in the present study. The pH of samples was slightly acidic (3.74 to 4.15), titratable acidity was low (0.094 to 0.155%) and sensory attributes (over all acceptability) were wide variable (5.9 - 6.69). The optimized selected commercial "mekmeko" tea obtained pH(4.05), titrable acidity(0.069%) ,acceptable sensory results (6.69), acceptable highest content of zinc (0.069 mg·L−1), magnesium (2.12 mg·L−1), potassium (62.34 mg·L−1),iron (0.28 mg·L−1) and calcium (17.19 mg·L−1). The nutrceticual property of optimized commercial "mekmeko" tea obtained acceptable highest content in total phenols (211.38 mg·L−1), total flavonoids (62.98 mg·L−1), total catechins (409.67 mg·L−1), and total anthocyanins (581.97 mg·L−1). Optimized "Mekmeko" teas showed the highest antioxidant capacity levels in DPPH (718.41 µmolTE·L−1), ABTS (607.62µmolTE·L−1), and FRAP (953.81µmolTE·L−1) assays. The results obtained in the present work give information to consumers for choosing flavored-colored ready-to-drink "mekmeko" tea based on the physicochemical, nutritional and nutraceutical properties.

Key words: *mekmeko tea, optimized, physicochemical, nutritional and nutraceutical properties*

Introduction

A spice may be defined as "any dried, fragrant, aromatic, or pungent vegetable or plant substance, in the whole, broken, or ground form, that contributes flavor, whose primary function in food is seasoning rather than nutrition, and that may contribute enjoyment or spiciness to foods or beverages" Although spices have been important for centuries in food preparation throughout the world, we see considerable difference in the patterns of spice use among cultures and countries. The indigenous knowledge and use of plants as spices and condiments (culinary use) is as old as the history of mankind [5-9] Achinewu et al. [10] pointed out that plants used as spices and condiments are usually aromatic and pungent. Iwu [13]; Billing and Sherman [18] had also reported that spices and condiments owe these properties to the presence of varying types of essential oils. Many authors also mentioned the antiseptic and the preservative properties of these plants to the possession of the essential oils such as what is reported in the work of Macmillan [7]. Dziezak [11] again indicated the rich presence of essential oils and oleoresins determine the aromatic, flavoring, coloring and pungent properties of spices and condiments.. ([14-15] Ethiopians have a long tradition of using spices and condiments for culinary as well as medicinal purposes. Here spices are important ingredients of foods and beverages of many ethnic cultures that are usually consumed in relatively large quantities and therefore can have significant health effects on a large sector of the Ethiopian population .[48-50]

Ethiopia as an ancient country has its own unique tastes and cultural inclinations. One that stands out is the Ethiopians love of spices. Almost all the food and drinks taken by Ethiopians in their daily routine consists of one or more spices, herbs, etc. Ethiopia's spices have a sort of mysterious nature about them, as they are not commonly found around the globe.[33] The various spices, herbs and roots Ethiopians use are mostly endemic to Ethiopia and it appears as if only the Ethiopians themselves possess the key that unlocks its many benefits. Today, with many Ethiopians living abroad, there is a large and growing market for exporting spices, as they still show an avid interest in the spices of their childhoods.[41] Ethiopians have been cultivating spices and herbs for a very long time now and the dedication and skill taken to prepare them is evident in the final product. The country today produces as much as 50 spices of the 109 spices that are listed by the ISO or the International Standard Organization for Standardization. Ethiopia is now mainly cultivating spices on a very small scale compared to her other agricultural products. The systems are all rain fed and cultivated from the wild, hence one could say that almost all Ethiopian spices are organic. This has helped them market their spices on the international market as the West has become very interested in healthy foods and spices. Spices and herbs have many applications, some of which are as a natural ingredient for the food industry, as well as being used by the cosmetics and pharmaceutical industries. These spices are commonly used throughout Ethiopia for medicinal, as well as, for culinary purposes. Spices and condiments are plant parts or plant products, which are mostly used for seasoning, flavoring and thus enhancing the taste of foods, beverages and drugs. [1-5]

Spices are aromatic plant products used in flavoring foods and beverages. The unique aroma and flavor of a spice is derived from compounds called photochemical which evolved in plants to enable them to protect against herbivores, insects and vertebrates, fungi, pathogens and parasites. [48-49] Different herbs, spices and condiments traditionally grow in various parts of Ethiopia. Its higher interior plains, cool nights and long growing season i.e., in general its diverse climate and soil type provide an abundant variety of food. Ethiopia is either a primary or a secondary center of origin of spices like Kororima (Aframomumcorrorima), Long pepper (Pepper longum), Black cumin (Nigella sativa), Bishop's weed (Carumcopticum), Coriander (Coriandrumsativum), Thyme (Thymus schimperi), Mekmeko (Rumex abyssinicus) and Fenugreek (Trigonellafoenumgraecum). (Ewnetu and Derseh, 2006) Spices have been extensively used in history for flavoring and seasoning foods, beverages and for medicinal purposes.[3] Therefore here one can understand that the culinary, medicinal as well as preservative use of herbs, spices and condiments has a long history in the world. A piece of the root (rhizome) of Rumex abyssinicus is dried, chopped and boiled in water and is added to flavor tea or can be drunk alone as tea. This is either to provide flavor or for its medicinal purpose. [34]

Amare Getahun [34] wrote about culinary use of R. abyssinicus. He explains that the cooked tuber of the plant is used to make a dye that gives yellow coloration to the clarified butter or other spice preparations. Rhizome of R. abyssinicus is pounded and added to tea or coffee and drunk. This is done to treat headache .Similar results have been described in the work of Tesfaye. [37] But here the part of the plant used to treat headache is different from what is reported by the local community in the study area. In the study area the bulb (rhizome) is used to relieve the problem but in Tesfaye [37], the leaf is used instead. The mode of administration of the remedy is also different. Mathewos and Agize [43] mentioned the medicinal value of R. abyssinicus and its refreshment in humans. Health benefits of "Mekmeko" that contains phytochemical to reduce blood sugar, anti diabetics, prevent blood pressure& Cancer, has anti diarrhea and anti microbial effect and also rich minerals and vitamins .[44] Even though spices produced in Ethiopia, there are no processing technologies; value additions and small scale processing industries are not yet available. Therefore the market demand at locally is minimum and the awareness of this spice to use is minimum because of un known its function .This implies most of organic spices in Ethiopia underutilized and its productivity is low because of lack technologies i.e. value addition.

In this study would optimize the mixing ingredients and evaluated the physicochemical, nuterucetical and sensory attributes

Materials and Methods

Sample collection and preparation

Rumex abyssinicus, spices additives ('Kerefa', 'Kirunfud'& 'Hel') sample were collected from local market and in the highlands and low lands of Amhara region. After collecting, "Mekmeko' and other spice additives samples were washed and dried. Then dried samples were milled as tea standard of course size as powder form.

Tea formulation and processing

Different formulations were used for 'Mekmeko' tea making (Table 1). The control tea(CST) was made using for comparable to the commercial one and 'Mekmeko'(100%, 75%,50%,25%) with that of other spice additives mixture . The blending samples were stored and sealed polyethylene bags at room temperature until they would analyze.

Table1. Formulations of 'Mekmeko' Tea prepared with other spices additives powder.

No	Ingredients	
	'Mekmeko' (%)	Spice additives ('Kerefa','Kurunfud','Hel'(%)
1	100	0
2	75	25

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3	50	50
4	25	75

Determination of Physicochemical Properties

pH and Titrable acidity (TA) determination: For the measurement of pH and titratable acidity, 10 mL of sample was diluted with 40 mL of distilled water, and the pH would read. After that, samples was titrated with 0.1 M NaOH to a pH of 8.2 (citric acid as predominant) using a Mettler-Toledo automatic T50 titrator (Greifensee, Switzerland) according to the Association of Official Analytical Chemists methods (AOAC, 2008).

Determination of Mineral Composition

Mineral analysis was done based on AOAC, 2008 methods using an Agilent Atomic Absorption 240FS spectrometer (Santa Clara, United States). Briefly, 5 mL of 3 M.HCl was added to 50 mL of tea sample and digested-evaporated at boiling temperature until 20 mL of sample will obtain. Afterwards, samples was filtered and used for mineral analysis.

Determination of Nutraceutical Properties

Total phenols, Total flavonoids, Total catechins and Total anthocyanins were carried out according to Niño-Medina et al. and AOAC, 2008. The content of total phenols was determined based on the reaction of Folin–Ciocalteu reagent. Briefly, 0.2 mL of tea sample was placed in 2.6 mL of distilled water, oxidized with 0.2 mL of Folin–Ciocalteu reagent, and after 5 min, neutralized with 2 mL of 7% Na2CO3 solution. The reaction was left for 90 min, and finally, the absorbance of sample was measured at 750 nm. Gallic acid was used as the standard (0 to 200 mg·L−1), and the results was expressed as milligrams of Gallic acid equivalent per liter of sample (mgGAE·L−1).

The content of total flavonoids will evaluate based on the reaction of aluminum chloride. Briefly, 0.2 mL of phenolic extract will place in 3.5 mL of distilled water, followed by 0.15 mL of 5% NaNO2. After 5 min, 0.15 mL of 10% AlCl3 will added, and 5 min later, 1.0 mL of 1 M.NaOH

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will add. Reaction will leave for 15 min, and finally, the absorbance of sample will measure at 510 nm. Catechin will use as the standard (0 to 200 mg·L−1), and the results will express as milligrams of catechin equivalents per liter of sample (mgCatE·L−1).

The content of total catechins was determined based on the reaction of vanillin-H2SO4. Briefly, 0.25 mL of phenolic extract was mixed with 0.65 mL of 1% vanillin solution and 0.65 mL of 25% H2SO4 (both dissolved in methanol). Reaction was leave for 15 min at 30°C, and finally, the absorbance of sample was measured at 500 nm. Catechin was used as the standard (0 to 200 mg·L−1), and the results was expressed as milligrams of catechin equivalents per liter of sample (mgCatE·L−1).For anthocyanins content, 200 µL of tea sample was mixed with 10 mL of ethanol-HCl (85 : 15 v/v, pH 1, 4°C) and shaked at 200 rpm for 30 min. Afterwards, the sample was centrifuged at 1000 rpm for 15 min, and finally, 3.5 mL of sample was measured at 535 nm. The content of anthocyanins was reported as milligrams of cyanidin-3-glucoside (C3G) per liter of sample (mgC3GE·L−1) using the following formula:

$C=(A/e)*(V/1000)*(1/\text{weight of sample})*10^6$ …………eqn-1

Where C = concentration in mgC3GE·L−1, A = absorbance of sample, ε = molar absorptivity (C3G = 26965 cm−1·mol−1), V = volume of sample, and MW = molecular weight of C3G (449.2 g mol−1).

Sensory Evaluation of Tea Samples

Sensory evaluation was carried out using a 15 main panelist to assess the organoleptic attributes

of the tea samples. The organoleptic attributes assessed were; the taste, the aroma, the flavor, the color, and the overall acceptability. The panelists were trained and selected randomly from the students of the university from Food process engineering department. They were made to carry out the organoleptic assessment under controlled environment to avoid biased results The panelists will be instructed to rate the breads based on 7-point hedonic scale ranging from 1=liked extremely to 7=disliked extremely.

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Statistical Analysis

For the analysis of the data, a one-way analysis of variance (ANOVA) was performed using SPSS software version 16.0. When significant deference's was observed, a mean comparison was made by the Tukey multiple range procedure ($p \leq 0.05$).

Result and Discussion

Physicochemical Parameters

The pH and titratable acidity are analytically determined in separate ways, and each has its own particular impact on food quality [16]. Titratable acidity is a better predictor of the impact of acid content on flavor of food, and the pH is a better predictor of the ability of a microorganism to grow in a specific food [17]. On the other hand, sensory attributes are the first notable characteristic of a food and often predetermines our expectation. Natural and synthetic colors play several roles in foods and beverages. There were statistical differences between tea samples in physicochemical parameters (Table 2). The values of pH in tea samples were slightly acidic and ranged from 3.74 to 4.15 while titratable acidity ranged from 0.0094 to 0.155 %.(Table 2) The results of the present study show higher pH values than data reported by Lunkes and Hashizume [19], whom analyzed 11 commercial ready-to-drink flavored teas available in Brazil market and found pH values ranging from 2.89 to 3.41. In addition, the titratable acidity reported by these authors was higher than our observations, since they found values ranging from 0.193 to 0.325%. The differences in the pH and titratable acidity values between these authors and our study are attributed to the fact that commercial available ready-to-drink teas contain citric acid as acidifier and sodium citrate as pH stabilizer to extend the shelf life of product. Therefore the selected optimized mekmeko tea in treatment two (75% mekmeko tea) had pH value, 4.05 and Titrable acidity, 0.069.

Table 2 Physicochemical property of ready-to-drink flavored-colored commercial "mekmeko" teas

No.	Treatment No.	pH	Titrable acidity (%)
1	1	4.15±0.02[a]	0.09±0.003[b]
2	2	4.05±0.06[a]	0.069±0.05[b]
3	3	3.95±0.034[b]	0.16±0.003[a]
4	4	3.82±0.1[c]	0.18±0.004[a]

*****Means ±SD with same alphabets in the same vertical row are no significantly different ($p \leq 0.05$)*

Mineral Composition

Minerals are crucial for the interaction between genetic and physiological factors. If a dietary deficiency of these elements exists, it will lead to physiological and structural abnormalities that are preventable and which may be reversed by administration of the element. Calcium, magnesium, sodium, potassium, iron, and zinc are essential elements, while copper and manganese are trace elements [21].

There were statistical differences between tea samples as in minor and as in major elements (Table 3). The tea samples among treatments ranged Fe (0.38-0.091), Zn (0.09-0.04), Ca (18.96-12.84), K (64.51-20.66) and Mg (2.24-0.28) and statically significant different as showed in table 3. The mineral composition of Tea in treatment 1 obtained the highest content of manganese, zinc, magnesium, potassium, and Calcium, while treatment 4 obtained the lowest.

Table 3: Mineral composition of ready-to-drink flavored-colored commercial “mekmeko” teas

No.	Treatment no.	Fe (mg·L−1)	Zn(mg·L−1)	Ca(mg·L−1)	K(mg·L−1)	Mg(mg·L−1)
1	1	$0.38{\pm}0.06^{a}$	$0.09{\pm}0.004^{a}$	$18.96{\pm}0.79^{a}$	$64.51{\pm}1.40^{a}$	$2.24{\pm}0.1^{a}$
2	2	$0.28{\pm}0.06^{b}$	$0.07{\pm}0.0006^{b}$	$17.19{\pm}0.34^{ab}$	$62.34{\pm}0.39^{a}$	$2.12{\pm}0.006^{b}$
3	3	$0.18{\pm}0.005^{c}$	$0.05{\pm}0.001^{c}$	$14.98{\pm}0.11^{bc}$	$44.92{\pm}1.24^{b}$	$1.05{\pm}0.04^{c}$
4	4	$0.09{\pm}0.013^{d}$	$0.04{\pm}0.001^{d}$	$12.84{\pm}0.53^{c}$	$20.66{\pm}0.29^{c}$	$0.28{\pm}0.004^{d}$

**Means ±SD with same alphabets in the same vertical row are no significantly different ($p \leq 0.05$)*

A wide range of selected micronutrient in made tea has been found in literature. The difference in micronutrient content of made teas could be attributed to the tea produced in different growing areas of the world having high variation in micronutrient contents, which resulted in differential micronutrient uptake by tea plants [22]. There is no literature available for comparison with the current report as there are no studies on the evaluation of mineral content of commercially ready-to-drink teas. Nevertheless, several studies have evaluated the mineral composition of prepared tea infusions. Reto et al. [23], prepared tea infusions by boiling 1.5 g of green tea in 250 mL of boiling water for 10 min obtaining levels of calcium (1.9 to 3.5 mg·L−1), potassium (92 to 15 mg·L−1), sodium (35 to 69 mg·L−1), and iron (0.02 to 0.12 mg·L−1). These values were lower than the mekmeko teas analyzed in the present study, but the content of manganese (0.5 to 1.9 mg·L−1) reported by these authors was higher than all our samples.

Nutraceutical Properties

Phenolic compounds are receiving interest because of the recognition of their antioxidant properties, their abundance in the human diet, and their probable prevention of several diseases associated with oxidative stress [25]. In addition, it is of great interest from consumers, nutritional experts, and food science researchers to know the antioxidant capacity of the foods that we consume [26].

There were statistical differences between tea samples in all phenolic evaluations (Table 4). The range values of total phenols, total flavonoids, total catechins, and total anthocyanins were 150.190 to 214.127 mgGAE·L−1, 20.353 to 72.383 mgCatE·L−1, 165.00 to 632.27 mgECat·L−1, and 235.21 to 635.12 mgC3GE·L−1, respectively. It was not a surprise that all samples were especially rich in total catechins as these compounds are the major phenolic group in tea infusions, accounting for 40% of total water-soluble solids in tea infusions [4]. The content of total catechins was higher than the content of total phenols, and although this behavior is very rare, it has been reported previously in some legumes by Xu and Chang [27] and Zia-Ul-Haq et al. [28]. The explanation of this behavior is that acid treatment of vanillin-HCl method has an effect on depolymerization of catechins, and they are quantified in more quantity than the phenolics quantified by Folin–Ciocalteu method where phenolics are not depolymerized.

Table 4: Nutraceutical properties of ready-to-drink flavored-colored commercial “mekmeko” teas

No	Treatment no.	Total phenols(mgGAE·L−1)	Total flanavoids(mgGAE·L−1)	Total chachins(mgGAE·L−1)	Total antocyniens(mgGAE·L−1)	ABTS(μmolTE·L−1)	FRAP(μmolTE·L−1)	DPPH(μmolTE·L−1)
1	1	214.13±0.99[a]	72.38±7.03[a]	632.27±8.62[a]	635.12±5.37[a]	1013.83±31.62[a]	1169.44±86.48[a]	1029.04±59[a]
2	2	211.38±1.07[a]	62.98±2.62[b]	409.67±18.31[b]	581.97±7.51[b]	607.62±66.95[b]	953.81±75.69[b]	718.41±118.66[b]
3	3	202.49±3.87[b]	43.26±7.23[c]	230.66±21.72[c]	362.78±41.76[c]	431.08±22.38[c]	455.88±23.11[c]	504.35±33.142[c]
4	4	150.19±4.56[c]	20.35±2.06[d]	165±0.45[d]	235.21±9.99[d]	231.90±0.79[d]	147.41±0.53[d]	117.74±0.67[d]

**Means ±SD with same alphabets in the same vertical row are no significantly different ($p \leq 0.05$)*

Anthocyanins are not a phenolic group presented naturally in tea; nevertheless, they were quantified in all samples. This is because of the use of exogenous anthocyanins that give the characteristic color of each flavored-colored tea.. There were statistical differences between tea samples in all antioxidant capacity evaluations (Table 4). The values of antioxidant capacity were 117.74 to 1029.04 μmolTE·L−1, 231.90 to 1013.83 μmolTE·L−1, and 147.41 to 1169.44 μmolTE·L−1 in DPPH, ABTS, and FRAP, respectively. Treatment 4 and treatment 1 were the lowest and highest in antioxidant capacity, respectively, in the three assays tested. The antioxidant capacity among the treatment was significantly different but reduced in treatment 1 to treatment 2.

To the best of our knowledge, few studies have reported the phenolic content and antioxidant capacity of commercial ready-to-drink teas. For instance, Kodama et al. [29] analyzed the phenolic content and antioxidant capacity of four ready-to-drink commercial teas available in Brazil, and they reported values around 1050 to 1705 mgCatE·L−1 and 140,000 to 545,000 μmolTE·L−1 in total phenols and DPPH antioxidant capacity, respectively, being these results greatly higher than those of ours. In addition, Chen et al. [30] studied the catechin content of 14 canned and bottled green tea drinks by HPLC, and they reported values from 0.9 to 341.7 mg·L−1 which are higher content found in our samples.

Other studies have reported higher values than ours, and this is because teas are prepared directly from tea product at laboratory scale without the thermal process (sterilization) and dilution that is carried on the commercial industry of ready-to-drink tea.

Although the polyphenols content in food label is not mandatory in the Official Mexican Standard for food labeling (NOM-051-SCFI/SSA1-2010), all the analyzed teas reported the content of these compounds in their labels, and this is may be to make an impact on the consumers about the functional food ingredients in the product.

Sensory evaluation

The commercial mekmeko tea among the treatments just after preparation were awarded sensory score of Tate, flavor, color and overall acceptability by the panelist were showed in below table. The sensory scores of treatment 2&3 were significantly different with the advancement of treatments of 1&4. The lowest sensory scores was observed in treatment No. 4 which is significant difference with treatment 2&3 whereas in treatment No.2 was maximum and no significant difference with Treatment No. 3 but significant difference with Treatment No. 1&4. Even though treatments 2&3 were statically not significant difference but the panelist were selected treatment "2" that is 75% of mekmeko ingredient mixed with 25% of spices. The sensory scores are given below in Table 5.

Table 5 sensory analysis of ready-to-drink flavored-colored commercial "mekmeko" teas

No		Sensory attributes			
	Treatment No.	Taste	Flavor	Color	Over all acceptability
1	1	5.86±0.89^{b}	6.25±0.17^{a}	6.25±0.86^{a}	6.0±0.73^{b}
2	2	6.83±0.96^{a}	6.79±0.83^{a}	6.56± 1.06^{a}	6.69± 0.77^{a}
3	3	6.44±0.63 ^{a}	6.63±0.13^{a}	6.44±0.51^{a}	6.56±0.51^{a}
4	4	5.56± 0.51^{b}	5.69±0.12^{b}	5.7500±0.45^{b}	5.69±0.48^{c}

**Means ±SD with same alphabets in the same vertical row are no significantly different (p≤0.05)*

Formulation and optimized of mixing flavored ingredients with mekmeko tea

In the optimization of "mekmeko" tea beverage formulation mainly the quantity of spices in equal amount("Kurifund", "Hel", "Kerefa") in mekmeko tea was optimized based on sensory evaluation. The sensory evaluation was based on four parameter color, taste, flavor and over all acceptability. For the optimization process different ratio of "mekmeko" with equal amount of spices (100%, 75%,50% 25% mekmeko) were employed. The average score obtained by each ratio are given in above table 5, in which the Treatment No. 2 (75% of "mekmeko" and 25% equal amount of spices("Kerefa", "Kurufund", "Hell") obtained the best scores.

Conclusions

Because of the increasing interest of consumers in the consumption of mekmeko tea powder teas with different flavors, it is necessary to know about their physicochemical and nutrition characteristics. There were differences between commercial "mekmeko" teas powder samples in physicochemical parameters, mineral composition, and nutraceutical properties. The pH values of tea samples were slightly acidic with values around pH 4.05; titratable acidity was low with values near to 0.1% in the selected treatment (75% mekmeko tea). the highest content of iron, zinc, magnesium, potassium, and calcium obtained in selected treatment.. In addition, the highest content in total phenols, total flavonoids, total catechins, and total anthocyanins were obtained in selected treatment(2) i.e 75% "mekmko" ingredients and showed the highest antioxidant capacity in DPPH, ABTS, and FRAP assays.. Finally, the results obtained in the present work give information to consumers for choosing commercial "mekmeko" tea powder based on the physicochemical, nutritional, and nutraceutical properties.

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