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Original Article

Investigation on Natural Color Extraction from Black Tea Waste

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Abstract

Tea is a source of natural color. Extraction of colored as a byproduct will make tea more remunerative. This study suggested the optimal conditions for natural color extraction from black tea waste. Tea manufacture waste was used in different concentration of solvent ethanol: water (50:50, 30:70, 10:90, and 0:100), time (30, 60, and 90 min) and temperature (20, 50, and 80°C). Factorial experiment with three factors (solvent, time and temperature) was performed in a completely randomized design with three replications. Extracted color was used in jelly compared to commercial color. According to the results, the total color extracted from 50:50 ethanol: water at 80°C was more than the other conditions ($p \leq 0.01$). In 80°C, time has no significant effect on color extraction in different solvent ratios. Extracted color had antioxidant activity in compared to commercial color. The taste of jelly colored by extracted color was no significant difference in compared with the jelly colored by commercial color ($p \leq 0.05$). Although the difference in outward appearance was easily detected by panelists, they preferred tea extracted color jelly in compared to commercial colored.

Keywords: Black tea waste, Color extraction, Jelly, Sensory evaluation.

Introduction

Tea plant is an evergreen shrub or small tree of the *Camellia* genus, native to China, with dark green shiny leaves and white flowers. *Camellia sinensis*(L.)Kuntze and *Camellia sinensis* var.*assamica* (J.W.Mast.)Kitam.are the two varieties most commonly used for the production of different types of tea. Freshly prepared infusion of dried leaves is a beverage consumed on a daily basis worldwide [1]. Flavonoids, flavonols and phenolic acids make up approximately 30% of dried *Camellia sinensis* by weight. Most of the polyphenols present are flavonols commonly known as catechins, with epicatechin and its derivatives being the most predominant forms [2]. Tea plant has provided a desirable beverage for centuries but it is also used as a colorant. Catechins are basically colorless, odorless soluble substances having a low molecular weight [3]. During fermentation in the formation of black tea, oxygen is absorbed by the catechins which are converted to the corresponding r-quinones. These quinones form dimers and further complex polymeric thearubigins. These thearubigins are the main components of black tea and mainly responsible for strength and color [4]. Both green and black tea powder and tea concentrates could be used as a natural color additive in the preparation of different tea-based food products such as confectionary and bakery items [5]. The compounds responsible for tea color are theaflavins (yellowish brown), thearubigins (reddish brown), flovonol glycosides (light yellow), phaeophorbide (brownish), phaeophytin (blackish) and carotene (yellow) [3].

Over the past decade the use of natural colors in food and beverages has increased at a much greater pace than that of synthetic colors. There are a number of reasons for this development, which is based on both technological improvements as well as market trends. The natural colors like annatto, anthocyanin, chlorophyll and caramel are those that are most well known and most widely used by the food and beverage industries. Some of other colors

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are saffron, gardenia, carthamus, lycopene and onascus [4]. Natural colors are extracted from natural sources. Some studies have been conducted on the extraction of colors from food industry residues. Extraction of yellow color from orange peel [6], β-carotene and lycopene from tomato paste waste [7], and pink grapefruit homogenates [8] are some of them.

The review of studies on tea has shown no implications for human use as a beverage or a colorant. The toxicology of tea is similar to cocoa and no adverse effects for humans have been implied [4]. The tea polyphenols have a remarkable range of pharmacological activities too. The black tea extracts (theaflavin and its gallates) will make an additional contribution in antibacterial activity [9]. They are classified in "good antioxidant" compounds that strongly inhibit peroxide formation [10]. The antioxidant property of green and black tea as well as green and black soluble tea has been proved.

Some studies were done on conditional optimization of tea color extraction and its application [2,5], but a few report are existed on natural food color extraction from tea waste. Faidah and Estiasih [11] produced natural brown food colorant from the tea waste, by foam mat drying method extraction. Tea waste that is produced beside of tea manufacture is consisted of significant amount of brown polyphenols. Extraction of by-products and their transformation to value-added products will make tea industry more remunerative. While normally tea waste and low quality tea were used as source materials. It will not only make tea remunerative but also produce some new tea-based food products with high antioxidant value. In the other hand, it is important to understanding the factors that influence extraction of natural colors, and successful application in food. In this study, in order to achieve maximum total color, natural color was extracted from tea waste in different conditions of solvent, temperature and time. Extracted color was used in a colorless jelly (Aloe Vera) to compare with commercial color.

Material and Methods

Tea waste was obtained from Shahid Eslami Tea Factory in Lahijan, Iran. Tea waste after milling was packed and kept away from moisture before extraction.

Extraction of the Color

Tea waste was extracted in the distillation system using a solvent of water: ethanol with different ratios of 50:50, 70:30, 90:10 and 100:0 in 20, 50 and 80°C at 30, 60 and 90 minute. The ratio of the sample (tea waste) to solvent was 2:100. Then the extracts were cooled, filtered and evaluated for total color determination as Obanda*et al*. [12]. In this method five mL of extracted tea were pipette into 45 mL distilled water in a 100 mL conical flask. The solution was shaken well to ensure thorough mixing. The absorbance of this solution at 460 nm (A_{460}) was read against distilled water blank. The total color was calculated as: Total color $=A_{460nm}\times 10$

Data was analyzed in completely randomized design with factorial experiment and three factors (solvent, time and temperature) in three replications.

The extracted color with maximum total color was concentrated to 50% solvent by vacuum evaporator. The alcohol test was used to assure the absence of alcohol in the extract of color by Jones Reagent [13]. Then concentrated color was sterilized at 70°C for 1 minute [14].

Determination of Color Properties

Determination of color properties such as density, total solids, tannin, antioxidant activity and pH were performed. The density was measured by using a pycnometer at 20°C. The weight of empty and full pycnometer was measured once distilled water and sample [15].

Total solids were measured by remove moisture in a two-stage process. The sample was pre dried over a steam bath before drying in an oven. Total solids contents of sample was calculated by [15]: Total solids (%) = weight of dry sample /weight of wet sample × 100

The determination of tannins was done by a method based on formation of insoluble tannins salts with copper (II) cation. This includes extraction of tannins with boiling water, followed by precipitation with copper (II) acetate and filtering after 12 h. The final precipitate was dried to constant mass. The content of tannins was calculated from a proportion, considering the quantity of copper taken for analysis and quantity of copper (II) oxide bound by tannins [16].

The antioxidant activity was measured by DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay. The effect of sample on the content of DPPH radical were evaluated by a spectrophotometric method based on the reduction of a methanol solution of DPPH. One ml of sample in methanol was added to one mL of a 0.003% methanol solution of DPPH. After 30 min, the reaction mixture was monitored at 517 nm by UV/vis spectrophotometer. The scavenging ability was calculated as follows [17]: scavenging ability (%) = $100 \times [A_{control} – A_{sample} / A_{control}]$.

The pH was measured on 100 mL sample by pH meter after calibration [18].

Application of Extracted Color in Jelly

Natural extracted color and brown commercial color (purchased from local market) were added a colorless jelly (Aloe Vera, purchased from local market). The amounts of tea extracted color and commercial color was 10 and 1 mL respectively for preparation of same colored jelly.

Sensory Evaluation

A triangle test was used for determine a significant difference in the appearance of Aloe Vera Jelly, and a preference test was used to determine the preference of the consumer. Fifty people of Tea Research Center of Iran employees were participated in this test.

Triangle tests: The two different samples were presented to the panelists in sets of three. The three samples were presented in identical sample containers coded with 3-digit random numbers. All three code numbers on the samples presented to each panelist was different. Results were analyzed using a one-tailed binomial test for significance. The number of panelists correctly identifying the different sample was totaled and the total tested for significance using One-Tailed Binomial Test table at a probability level of 0.05% [19].

Preference Test: Preference tests allow consumers to express a choice between samples; one sample is preferred and chosen over another or there is no preference. The two samples were presented in identical sample containers coded with 3-digit random numbers. The samples were presented simultaneously in the order selected for each panelist. Results were analyzed using a 2-tailed binomial test. The number of judges preferring each sample was totaled and the totals tested for significance using Two-Tailed Binomial Test Table [19].

Results

Optimization of Color Extraction Conditions

The extracted total color (%) was significant different between extraction conditions in this experiment ($p \leq 0.01$). In other words, the different ratios of water and ethanol solvent, temperature and extraction time affect the extracted total color.

The interaction effects of solvent and temperature on 30, 60, and 90 minute are presented in figs. 1 to 3 respectively. Fig. 1 shows total color changes in different solvents and temperatures during 30 minute. The total color was increased with increasing ethanol content in the solvent and temperature. In solvent with 50% ethanol and 80°C, the maximum color extracted was 4.3% and 4.4% in 60 and 90 min, respectively (Fig. 2 and 3). In all three times of the extraction, a very small difference (0.1%) was observed between 10% and 0% ethanol at 20°C, and in other temperature it was a little more.

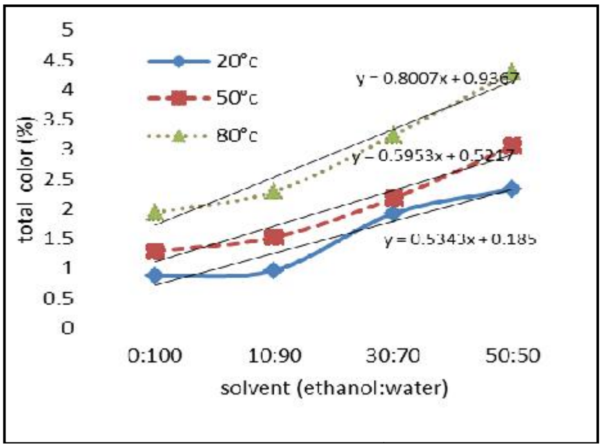

Fig. 1 Changes in total color extracted in different solvents and temperatures during 30 minutes

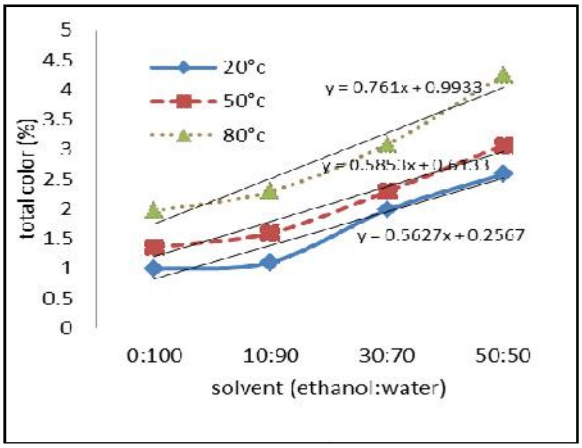

Fig. 2 Changes in total color extracted in different solvents and temperatures during 60 minutes

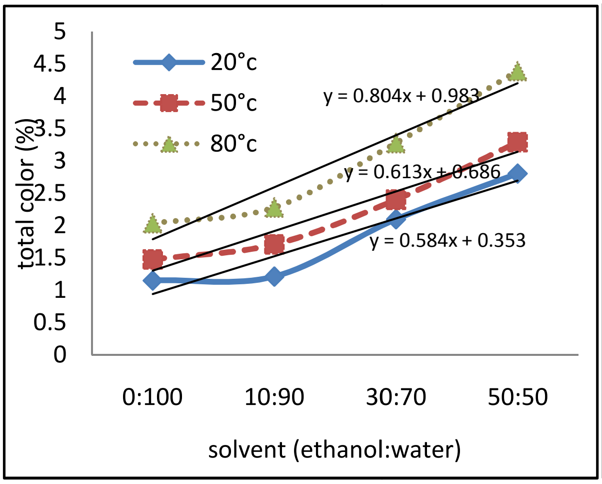

Fig. 3 Changes in total color extracted in different solvents and temperatures during 90 minutes

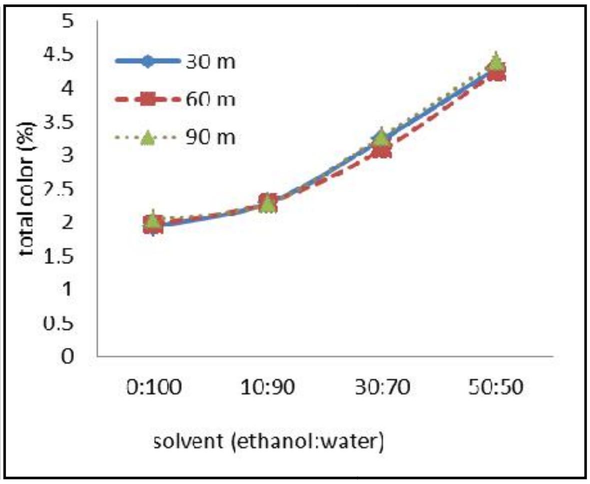

Fig. 4 Changes in total color extracted in different solvents and times at 80°C

Fig. 4 showed that in 80°C, time had no significant effect on color extraction in different solvent ratios. In other words, the prolongation of extraction time from 30 to 90 min did not affect the amount of color in 80°C.

Color Properties

A comparison between the physicochemical properties of extracted color with commercial brown color that is available in local market was given in Table 1. The total color in commercial color was about seven times the amount of tea extracted, but has no tannin and antioxidant activity. Therefore, replacement the tea extracted color to food in addition to coloring can create beneficial properties for consumer health. Total solid of commercial color was more than tea extracted color but the density of them has no substantial difference.

Sensory Evaluation

All of person in triangle test recognized the different between colors of samples. There was significant difference in the color of the samples according to the one-Tailed Binomial test table.

The results of preference test are presented in Table 2. Comparison of results with two-dimensional binomial test table showed that jelly with tea color was preferred by panelists in significant difference ($p\leq0.05$). The samples did not show any significant difference in taste ($p\leq0.05$).Although the difference in outward appearance was easily detected by panelists, they preferred jelly with tea color. Meanwhile the difference in taste was not significant and addition of tea color was not cased a distinct difference in the taste of jelly.

Table 1 Physicochemical property of tea color and commercial brown color

Type of color	Total color (%)	Antioxidant activity (%)	Tannin (%)	Total solid (%)	Density (g/ml)	pH
Extracted color	4.86	29.1	1.07	1.5	0.98	4.8
Commercial color	28.0	0	0	2.2	1.00	7.7

Table 2 Selection of panelists in the preference test

number of evaluators	selecting color		selecting taste	
	tea color	commercial color	tea color	commercial color
30	23	7	12	18
p-value	0.005*		0.342^{ns}	

* and ^{ns} respectively significant and not significant at $p\leq0.05$

Discussion

Black tea waste can be used to extract food color as a valuable by product. The optimum ratio of solvent for color extraction of tea waste was 50:50 ethanol: water at 80°C. The total color was 4.5% in this condition and it was about two fold of total color extracted at 20°C. Song [20] and Meterc [21] reported using 90°C for 10 minute and 80°C for 15 minute respectively for increasing the polyphenols extraction efficiency. Increasing the temperature could be softening the plant tissue and cause to release the polyphenols in the solvent. The colored compounds in tea include a very complex mixture of polyphenolic compounds [4], which are effectively segregated in this method. The greater slope of curves at 80°C (in Figs. 1-3) showed the increase in ethanol content of solvent has a greater effect on color extraction in comparison to other temperatures. Maximum polyphenols extraction efficiency was reported in microwave method by ethanol [22] and most extraction of phenolic compounds from grape seeds was reported by 1:1 ratio of ethanol: water as solvent [23].

It will be cost-effective to use 30 minute extraction in 80°C. Long time heating means energy consumption and expenses. In the other hand increasing in time might depredated the antioxidant compounds possibly because of some loss of phenolic compounds via oxidation and these products might polymerize into insoluble compounds [23]. Najafi*et al*. [24] reported the process of extracting soluble dry matter from black tea dust was slowed down after 30 minute.

Tea extracted color can be the most desirable option in food industry due to their nutritional and therapeutic properties, like antioxidant activity, without any change in taste. Baruah*et al*. [5] reported extraction of colored components of tea and incorporation of them into some food products will make tea more remunerative and produce some new tea-based food products with high antioxidant value. The acidic pH (4.8) of the extracted color indicated that it will be appropriate to add to the acidic food products (like candy). The alkaline pH can cause darkness in the color because of oxidation of polyphenol to dark compounds like theanaphthoquinone [25]. Tea extracted color should be utilized more because of their less coloring power in compared with commercial colors. It could be more concentrated for industrial food. Sensory evaluation showed jelly with tea color was preferred to commercial color in taste.

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