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Development of a Food Colourant Using Refused Tea Generated by Ceylon Black Tea Industry as a Substitute for Caramel Black (E, INS 150)

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Abstract: *Caramel colour is commonly used in food products such as bakery products, Soy bean sauces, ground sauces, soft drinks, ground molt, alcoholic beverages and in gravies and vinegar. According to the modern researches consumers of these beverages can be exposed to 4- methylimidazole (4-MEI), a potential carcinogen formed during its manufacture. The objective of this research study was to develop a refused tea based food colourant as a substitute for caramel colour. After selecting the proper raw materials(96.68% moisture , 0.52% Ash, 0.58% total solid) colour extraction and stabilization was carried out with carrageenan (0.6-1.0 %) and the behaviour of the product was analyzed with different pH values, concentrations and time – temperature conditions. Furthermore, viability of the developed colourant was studied with the commercially available caramel colour(E , INS 150) using structured sensory evaluation tests and colour comparison techniques . The initial pH value of the product was 4.9 and the developed colour can be used from 3 to 12 pH range effectively. And also the product can be used from 10YR 6/8 to 7.5YR 0.4/2 range in different concentrations. (0.05 to 3.0% v/v) without any significant difference in developed food colour and the commercially available caramel black colour. So the developed product is applicable for the food industry. Moreover the total polyphenol content of the product was 0.04718 mg/ml and Trance – 2 – hexenal, Cis - 3 - Hexenol, Linalool, Methyl salicylate, 2-phynylethanol, β - ionone have been identified as the main flavor compounds.*

Keywords: *Refused tea; food colour; polyphenols; Tea colour; flavour compounds*

I. Introduction

The term food colour has been defined as any substance that is added to food or beverage to change its initial color. People associate certain colours with certain flavours, and the colour of food can influence the perceived flavour in a wide range from candies to alcoholic beverages. For this reason, food manufacturers are adding synthetic food colours to their products. Caramel colour is one of the oldest and most widely-used soluble food colorings, which is made by a carefully controlled heat treatment of carbohydrates, generally in the presence of acids, alkalis, or salts, in a process called caramelization. It is fully oxidized than caramel candy and has an odor of burnt sugar and a somewhat bitter taste. Its colour ranges from pale yellow to amber to dark brown Generally caramel colour produced with Ammonium compounds (i.e., caramel color type IV). The use of these compounds to produce caramel colour can result in the formation of 4-methylimidazole (4-MEI). In recent years, evidence for the carcinogenicity of 4-MEI has raised concerns about uses of caramel colour type III and IV that may expose consumers to 4-MEI and increase cancer risk.

Tea extracts have gained popularity as ingredients in dietary supplements and functional foods. Epidemiological and animal studies reveal that tea is protective against certain cancers, cardiovascular diseases, and neurodegenerative diseases [1]. Tea has a complex chemical composition, containing over 2000 components. [2]

The Sri Lankan tea industry has a long history spanning close to a century and a half to become the third largest agricultural crop in the country [3] which provides over 1 million direct and indirect employments while generating significant amounts of foreign exchange [4]. In addition, the country accounts for 9% share of world tea production and about 19% of total global tea exports [5].

Sri Lankan tea industry annually produced around 320 million kilograms of made tea, according to the current statistics available. Out of the given production output, the country has manufactured approximately 95% black tea annually, which basically intended for export representing 32% of the global demand on orthodox black tea where Sri Lanka is still the market leader for orthodox black tea [6].

Refused tea is the waste produced during the manufacture of black tea. According to the tea control act No. 51 of 1957, the refuse tea is defined as sweepings, mature leaves, fluff, mature stalk or any other product (not being made tea) obtained in the process of manufacture of tea. In Sri Lanka, about 4 - 6% of the total product of made tea is refused tea [7].

The amount of refused tea produced depends on the method used in manufacturing of tea. Sometimes under the conditions where unsuitable leaves are available for manufacturing such as coarse plucking or under withered or over withered leaf are available, the percentage of refused tea could increased even to a level 8-10% [7]. There is a rising illegal market for refused tea and it has become a challenge for the goodwill of the sri Lankan tea market [8].

Yet not much studies has been carried out except, the use of refused tea as litter material for broiler chickens [9].Tea Refuse as a Material for Compost Production [10], Potential exploitation of refused tea as an alternative medium in mushroom cultivation [11], Effect of waste tea on the growth of young tea plants.

So the major objective of this research study was to utilize of the refused tea to make food colouring with low production cost. Because it is highly important if tea wastage can be reduced by developing a product by using them as a main ingredient. And also this will be a good opportunity to increase the national gross domestic product (GDP) while opening new markets as a tea producing country.

II. Materials and Methodology

2.0 Raw materials

According to the objectives refused tea which were collected at refused tea stores at plantations in Baddegama, Sri Lanka used as the major raw material to obtained the natural food colour as a substitute for caramel colour. Several raw material samples were used in different temperature- time combinations.

2.1 Extraction of natural food colouring by refused tea

The identified sample (510) was initially tested forAsh (AOAC 94546), protein (AOAC 960.52), crude fibre(AOAC 978.10) and moisture (oven drying method). Then 50 g of the aforesaid sample was boiled for 10 minutes and filtered through a grade 01 filter paper using suction filtration method.

2.2 Development of edible colouring agent for food industry

The filtrate was led to continually heat (85-90^{0}C) in a water bath until the brix valueof the filtrate 15^{0}Bx. There after the concentrated filtrate cooled under room temperature up to 65-75 ^{0}C and 1% of carrageenan (0.6-1.0 % based on the requirement) was added and vigorously stirred. Finally the product was stored in cleaned and dried glass containers.

2.3 Determination of stability of the developed product against concentration, pH, time and temperature.

As the introducing food color, the behavior of the developed product in different environment were analyzed..

2.4.1 Concentration vs colour

Initially a series of different product concentrations were prepared as 3, 2.5, 2.0, 1.5, 1.0, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.05 %. After that colour of the each solution was compared with the standard Munsell colour chart.

2.4.2 pH vscolour

Buffer solutions were prepared with different pH values (3,4,6,7,8,9,10,11,12) using Sodium hydroxide (PubChem CID – 14798) pellets and Citric acid (PubChem CID -311). Thereafter 100 ml of each buffer was taken and 2.5 ml of the developed product were added using a burette and mixed well before the color inspection.

2.4 Comparison of the developed food color with commercially available food colouring.

Caramel black colouring (E, INS 150) was identified as the compatible, commercial available food colour for the developed colour. A series of solutions were prepared with different concentrations for both colours and finally their colours were analyzed. Moreover the compatible proportions were identified for the developed product to obtain the same colour for commercial purposes. In addition to that sensory evaluation was also carried out with the final samples incorporated biscuits using commercially available product (587), developed product (743) and also with the control sample (289) at the Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenapura, Sri Lanka with the participation of 32 panelists. Finally data was analyzed using Mann Whitney test, Minitab 16.0 statistical software.

2.5 Identification of flavour compounds of the developed product.

Flavor compounds of the product were analyzed by using Gas Chromatography –GC (Agilent 7890A)referring GC-FID , Sri Lanka Tea Research Institute (TRI) method . [12].

2.6 Determination of total polyphenol content in developed product

According to the ISO 14502-1:2005E, colourimetric method, Folinciocalteu reagent was used to determine the polyphenol content of the product. After preparation of samples in order to the above method, absorbance values were obtained by using spectrophoto meter (Cary 50 / serial number – EL04033282,wave length- 765 nm) and total polyphenol content was calculated using following formula.

$$\mathbf{W_T} = \frac{(Dsample - Dintercept) \times Vsample \times d \times 100}{Sstd \times msample \times 10000 \times wDMsample}$$

D sample - is the optical density obtained for the sample test solution;
D intercept - is the optical density at the point the best-fit linear calibration line intercepts the y-axis;
Sstd- is the slope obtained from the best-fit linear calibration;
Msample- is the mass, in grams, of the sample test portion;
Vsample- is the sample extraction volume, in milliliters (50 ml for instant tea and 10 ml for leaf tea);
d - is the dilution factor used prior to the colorimetric determination (typically 1,0 ml to 100 ml, thus a dilution factor of 100);
wDM - sample is the dry matter content, expressed as a mass fraction in percent, of the test sample.

III. Results and Discussion

3.0 Identification of raw materials

Refused Tea was the major raw material and this is totally a waste product generated by Ceylon black tea industry. Average nutrition values for the raw material are as follows,

Table 1- proximate analysis results for the raw material

No	Nutrition Factor	Results	Ref. method
1	Moisture	96.67%	Oven drying method
2	Protein	-	AOAC 960.52
3	Fat	-	-
4	Crude fibre	-	AOAC 978 10
5	Total solids	0.52%	-
6	Ash	0.78%	AOAC 945.46

According to the table 01 there is no protein, fat, and crude fibre in a detectable level, but 0.52, 0.78% of total solids and ash available respectively while moisture content was 96.67%.
Moisture content may vary for different samples due to their storage conditions. Furthermore, Ash content reveals weather it is contaminated or not.

3.1 Identification of effective temperature – time relationship

The product intendent to use for the different purposes in food industry as shown in table 02.

Table 2 Temperature- time co-relationship

Temperature (°C)	Time (minutes)	Color	Munsell color code
30	3		10YR 6/10 #c8881a
	6		2.5YR 5/14
	9		2.5 YR 5/12 #c85a15
	12		2.5 YR 4/10 #a1414
60	3		10 R 3/8 #802c20
	6		10R 2/8 #221114
	9		10R 2/6 #581d1c
	12		2.5 YR ¼ #380e0b
100	3		2.5R 0.8/2 #2a1110
	6		110R ½ #2e1518
	9		7.5PB 0.2/20
	12		7.5 PB 0.2/20

Table 02 reveals that temperature and time harshly affected on the color of the product. From 3 to 12 minutes in 30^0C, the product characterized in light color range from 10YR 6/10 to 2.5 YR 4/10. Moreover 03,06, 09 and 12 minutes in 60^0C cause for 10 R 3/8 , 10R 2/8, 10R 2/6, 2.5 YR ¼ colors respectively. To obtain dark colours (2.5R 0.8/2 to 7.5 PB 0.2/20) the product were heated at 100^0C for 3 to 12 minutes. In addition to that the colourprofile of the product varies with the pH value as shown in table 03.

Table 3-Colourvs pH value

No	pH value	Color	Munsell color code
1	3.0		10YR 6/8 #c2893b
2	4.0		7.5YR 4/6 #87552b
3	6.0		5YR 3/8 #763600
4	7.0		5YR 3/8 #763600
5	8.0		5YR 4/8 #934f1f
6	9.0		7.5YR 3/6 #6a3d14
7	10.0		2.5YR 3/8 #7b3111
8	11.0		5YR 1/4 #371001
9	12.0		7.5YR 0.4/2

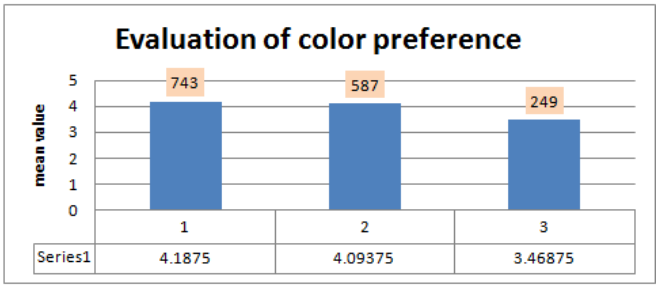
According to the table 03 there is a huge variance in colour by different pH environments. The initial pH value of the product was 4.9 and the colour has been changed from light (10YR 6/8) to dark (7.5YR 0.4/2) while increasing pH, from 3-12. Most of the coloured food products (RTS, Jam, Concentrated fruit juice..etc) are in an acidic conditions. So it is intended to use the developed colour for these products. In the other hand, bakery products, traditional food products (oil cake) are in high pH levels and it is possible to use this product to obtain the colour.

3.2 Comparison of the developed product with commercially available food colouring

3.3.1 Structured sensory evaluation

Five point hedonic scale structured sensory evaluation was carried out for the developed product to analyze the sensory attributes and consumer preference. Moreover commercially available caramel food colour was also used for the sensory evaluation as shown in following figures.

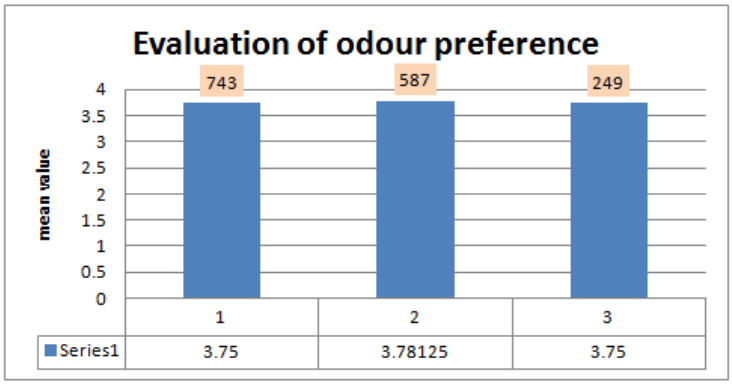
Respondents Preferencethe product colour.



Graph 1- Evaluation of Colour Preference

According to the graph 01 , Median difference between 743 and 587 was 0.0000 but within 95% confidence interval median difference was 0.0001 to 0.0000. Further P- value was 0.4340 which was greater than 0.05 (alpha value) and there was no significant difference between sample 743 and 587. So there was no statistically significant difference in colour for commercial caramel added biscuit and developed new colour added product. Furthermore median difference between 743 and 249 was 1.0000 and within 95% confidence interval median difference was 1.0000 to 0.9999 . P- value was 0.0000 which lower than 0.05 (alpha value). So there was a statistically significant difference in sample 743 (developed new colour added product) and 249 (controlled sample).

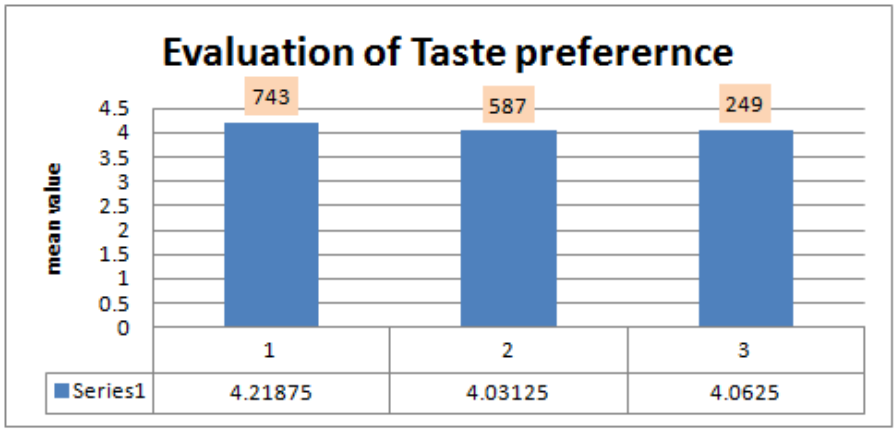
Respondent's preference for the Odour.



Graph 2 - Evaluation of Odour Preference

According to the graph 02 , Median difference between 743 and 587 was -0.0000 but within 95% confidence interval median difference was 0.0001 to 0.0000. Further P- value was 0.8563 which was greater than 0.05 (alpha value) and there was no significant difference between sample 743 and 587. So there was no statistically significant difference in colour for commercial caramel added biscuit and developed new colour added product. Furthermore median difference between 743 and 249 was 1.0000 and within 95% confidence interval median difference was -0.0000 to 1.0000 . P- value was 0.8020 which greater than 0.05 (alpha value). So there was no statistically significant difference in sample 743 (developed new colour added product) and 249 (controlled sample).

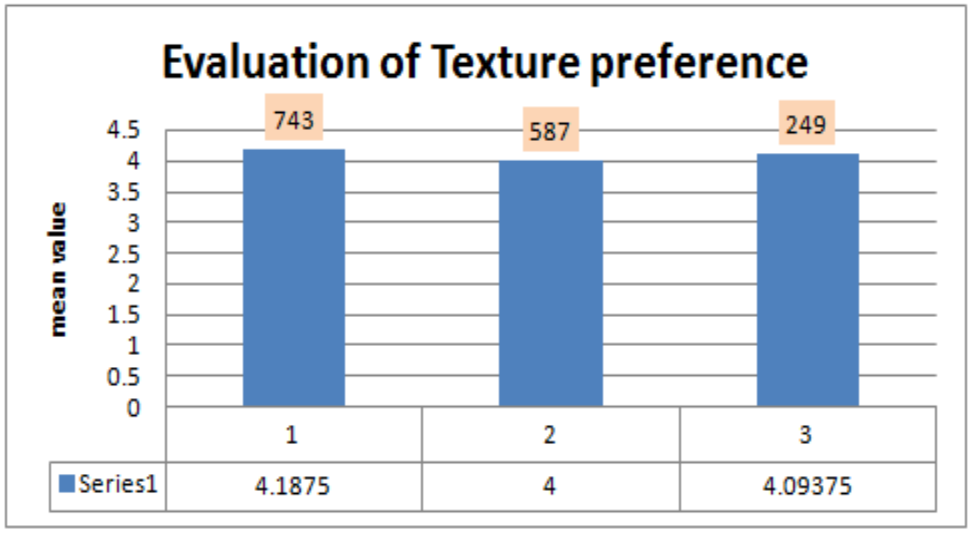
Respondent's preference in taste of the product



Graph 3- Evaluation of Taste Preference

According to the graph 03, Median difference between 743 and 587 was 0.0000 but within 95% confidence interval median difference was 0.0003 to 0.9999. Further P- value was 0.1582 which was greater than 0.05 (alpha value) and there was no significant difference between sample 743 and 587. So there was no statistically significant difference in colour for commercial caramel added biscuit and developed new colour added product. Furthermore median difference between 743 and 249 was -0.0000 and within 95% confidence interval median difference was -0.0000 to 1.0001 . P- value was 0.1582 which was greater than 0.05 (alpha value). So there was no statistically significant difference in sample 743 (developed new colour added product) and 249 (controlled sample).

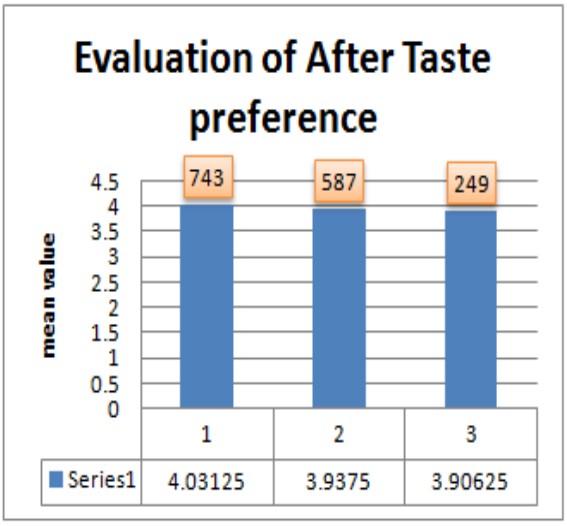
Respondent's preference in Texture



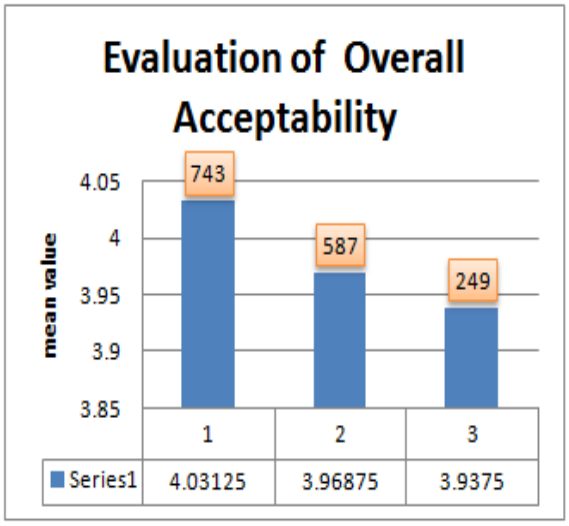
Graph 4- Evaluation of Texture Preference

According to the graph 04, Median difference between 743 and 587 was 0.0000 but within 95% confidence interval median difference was -0.0001 to 0.9998. Further P- value was 0.3169 which was greater than 0.05 (alpha value) and there was no significant difference between sample 743 and 587. So there was no statistically significant difference in colour for commercial caramel added biscuit and developed new colour added product. Furthermore median difference between 743 and 249 was 0.0000 and within 95% confidence interval median difference was 0.0000 to 0.9999 . P- Value was 0.6189 which was greater than 0.05 (alpha value). So there was no statistically significant difference in sample 743 (developed new colour added product) and 249 (controlled sample).

Respondents preference in After taste and Overall acceptability



Graph 5- evaluation of after taste preference



Graph 6- evaluation of overall acceptability

Graph 05 and 06 reveals that p-value of the sample 743 and 587was 0.6220, 0.7973 for After taste and overall acceptability respectively. So there was no significant different between 743 and 587 samples for above attributes. When comparing sample743 and 249, p-values were, 0.4807 and 0.5809 respectively for after taste and overall acceptability. So there was no significant difference in between sample 743 and 249 for after taste and overall acceptability.

3.4 Colour comparison between developed new product and commercial caramel colouring.

Colour comparison in between the developed colour and commercially available caramel colour are shown in table 04.

Table 4- Colour Comparison of Commercial Caramel Colour and Developed New Colour

Commercial caramel coloring			Natural Tea coloring		
Concentration	Observed color	Munsell color code	Concentration	Observed color	Munsell color code
0.001		2.5Y 5/6	0.2		2.5Y 6/8
0.03		5YR 4/8	0.4		5YR 5/10
0.04		2.5YR 3/8	0.5		2.5YR 4/8
0.05		2.5 YR 2/8	1.0		10R 3/8
0.06		5YR 1/4	1.5		10R 2/8
0.07		5YR 1/8	2.0		2.5YR 1/4
0.1		2.5 YR 0.6/2	3.0		10R 1/2

In order to table 04 a strong co – relationship can be identified inbetween the developed and commercially available colour. To obtain the colour (2.5Y 5/6) of commercial available colouring and to obtain colour (2.5Y 6/8) by developed new product , concentrations were 0.2 , 0.001% respectively. Also to obtain colour (5YR 4/8) by commercial colouring and the colour (5YR 5/10) by developed new product, while concentrations were 0.03 and 0.4% respectively.

3.5 Identification of flavour compounds of the developed product

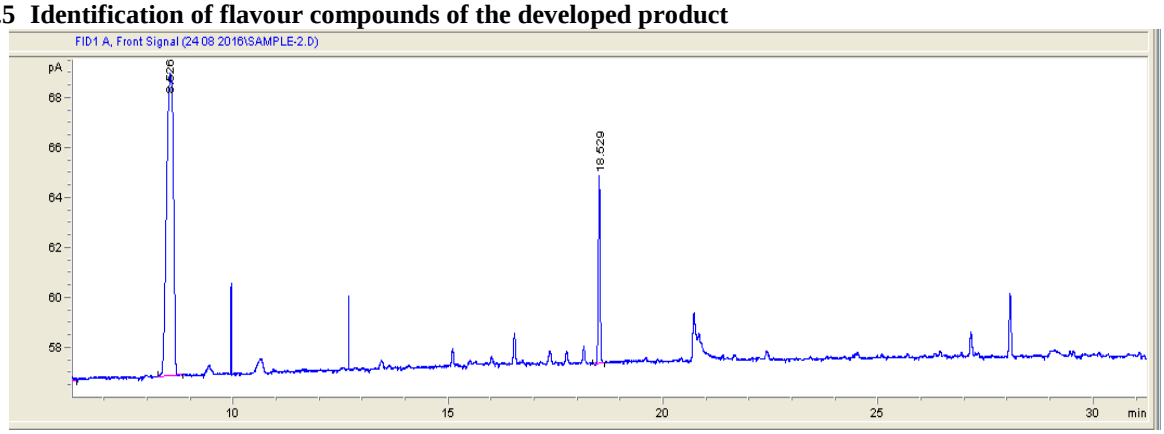


Fig- 1-GC spectrum of mix Standard

Developed edible colour was qualitatively analyzed for the flavour compounds by using the GC method. According to the GC report there were 6 compounds which were directly effect on the flavour detected has mentioned in figure (1).

According to the figure 01 the final product consist with (Trance – 2 – hexenal, Cis - 3 - Hexenol, Linalool, Methyl salicylate, 2-phynylethanol, β - ionone) . Among them2-phynylethanol(28.226) and Linalool (18.529) were present in a higher amount.

Flavour compounds were identified with reference of the standard curve drown initially and all most all the compounds were highly volatile and decompose with the time.

3.6 Determination of total polyphenols in the developed product – Colourimetric method using Folin-Ciocatalteu reagent

Initially standard curve was prepared using standard solutions (Gallic acid) to determine the total polyphenols. The standard curve as well as the absorption values of the samples as shown below.

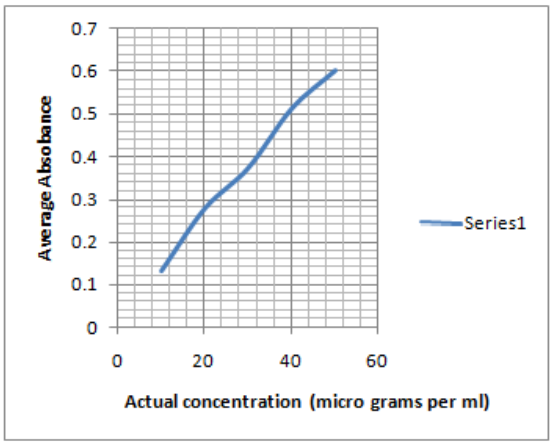


Fig - 2 - Standard Gallic acid curve

Table 5-Sample Absorptions Relative to Gallic Acid

Sample	Dilution factor	Absorption	Specific wave length
Blank	-	0.0991	765.0 nm
1	1250	0.5825	765.0 nm
2	1250	0.5798	765.0 nm

According to the table (5) absorption values were 0.5825 and 0.5798 at the specific wave length 765 nm. In order to the Folin- Ciocatalteu method the total polyphenol content of the final product was 0.04718 mg/ml.

IV. Conclusions

- Refused black tea which was having 96.68% moisture , 0.52% Ash, 0.58% total solids could be used to develop the edible food colour as a substitute for caramel black (E , INS 150) colour.
- The final brix value was 15^0Bx and 1% of carrageenan (0.6-1.0 % based on the requirement) was identified as the thickener for the developed food colour.
- The initial pH value of the product was 4.9 and the developed colour can be used from 3 to 12 pH range effectively.
- This study reveals that the product can be used from 10YR 6/8 to 7.5YR 0.4/2 range in different concentrations. (0.05 to 3.0% v/v).
- There was no significant difference observed among the developed food colour and the commercially available caramel black colour. So the developed product is applicable for the food industry.
- The total polyphenol content available in the product was 0.04718 mg/ml and Trance – 2 – hexenal, Cis - 3 - Hexenol, Linalool, Methyl salicylate, 2-phynylethanol, β - ionone were the main flavour compounds detected in the product.

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