ACCEPTABILITY OF OREGANO AND LEMON GRASS READY TO DRINK TEA

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ABSTRACT

Ready to drink (RTD) herbal tea beverage has gained popularity and conquered tea markets due to increased awareness of health benefits from polyphenols. Oregano and lemongrass could be considered as a potential functional food ingredient due to its health benefits. Thus, this study aims to utilize oregano and lemongrass into RTD tea and to determine its acceptability. The most acceptable formulation of the product was tested for: physico-chemical properties (brix, vitamin C, pH) and proximate properties (moisture, fat, ash, protein and carbohydrate) and microbiological tests using official methods. Results of proximate analyses showed that Oregano-lemongrass RTD contain 94.6% moisture, 5.4% carbohydrates, 0.02% ash, 5.09 pH, 9.8 brix and 0.01 vitamin C in mg/100mL. The microbial analysis results complied with the microbial standards of FDA in 2013 for Herbal ready to drink products. The sample composed of 80% Oregano and 40% lemongrass extract was the most acceptable amongst the formulations.

Keywords: *Functional foods, Lemongrass, Oregano, Ready to drink tea (RTD)*

INTRODUCTION

Functional foods gained considerable interest in the food and nutrition industry for years (Siro *et al.,*2008). The practice of using nutritional knowledge in food product to improve the health of consumer forms the general concept of functional foods (Peressini and Sensidoni, 2009). Interests in the use of herb and spices proliferate from a commonly employed food ingredient to different types of food preparations, to becoming the main focus of utilization to improve product's nutritional quality since they contain a wide variety of compounds that have shown to have beneficial health effects.

Nowadays, the range of functional foods includes products such as baby foods, baked goods and cereals, dairy foods, confectionery, ready meals, snacks, meat products, spreads, and beverages (Ofori and Hsieh, 2013). In particular, beverages are by far the most active functional foods category because of (i) convenience and possibility to meet consumer demands for container contents, size, shape, and appearance; (ii) ease of distribution and better storage for refrigerated and shelf-stable products; (iii) opportunity to incorporate desirable nutrients and bioactive compounds (Sanguansri and Augustin 2009; Wootton-Beard and Ryan 2011; Kausar *et al.,* 2012).

According to Salao *et al.* (2005), people believe that tea, which is considered as the second world most consumed beverages in the world; have a lot of nutritional factor. Nowadays, different raw materials are being processed as alternative in producing other kind of tea. Different herbs with nutritional and medicinal value are being used and these herbal infusions are packaged as tea and enjoyed like tea and widely accepted to be called as "tea" even though the herbs do not come from authentic tea plant and herbal tea is gaining popularity all over the world. According to Kim (2008), RTD herbal tea beverage has gained popularity and conquered tea markets due to increased awareness of health benefits from antioxidant polyphenolics.

Oregano has many health benefits and a potential functional food ingredient. It is known to have strong anti-bacterial properties and has been used in traditional Philippine medicine for a long time. It is a known herbal medicine for its strong anti-oxidant properties. It contains a rosmarinic acid compound, thymol, and carvacrol that are responsible for its anti- inflammatory, anti-bacterial, anti-oxidant, anti-fungal and anti-viral properties. It also contains flavonoids, triterpenoids, sterols, vitamin C, and vitamin A.

Lemongrass (*Cymbopogon citratus* Stapf.), commonly known in the Visayan language as *tanglad*, has long been used in the Philippines for its many medicinal uses, such as for fever and as a diuretic. In the study of Puatanachokchai *et al.,* in 2002 they stated that lemongrass contains some components that may be cancer chemo preventive. Shah *et al.,* in 2011, identified terpenes, alcohols, ketones, aldehyde and esters as main compounds in

Cymbopogon citratus. Asaolu *et al.,* in 2009 analyzed the phytochemical constituents of the leaves of *Cymbopogon citratus* which showed that lemongrass contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids.

Oregano and lemongrass have been shown to have a lot of healthful benefits but are rarely used because they are not easy to consume. Thus, this study aims to utilize oregano and lemongrass into an innovative and convenient product as ready-to-drink tea. Also, this study aims (1) to know the suitable formulation for the oregano and lemon grass ready to drink tea, (2) to be able to know the acceptability of ready-to-drink (RTD) oregano and lemongrass tea among consumers, (3) to determine the proximate composition of RTD oregano and lemongrass tea, (4) to determine the physico-chemical characteristic of the developed product particularly it's total soluble solid (TSS), pH and vitamin C, (5) to determine the microbial composition of the developed product as a measure of its safety assessment, to conduct cost analysis of the product.

The significance of the study is to utilize the possible functional ingredient, the oregano leaves and lemongrass into new product. This study is vital to the ready-to-drink tea consumers, because it provides a new variety of flavor which is healthier and cheaper.

Theoretical Background

Theoretical Framework

The study discusses the possibility of oregano and lemongrass to be a potential functional ingredient as they contain a lot of healthful benefits and the utilized oregano and lemongrass ready-to-drink tea can be considered as a functional beverage. It also states the background of the raw materials that will be studied and utilize, the overview of main ingredient that will be used; oregano leaves and lemongrass, their health benefits and other uses. The related studies and information about the oregano leaves, mint leaves and ready to drink in local and foreign literature also the published and unpublished thesis are indicated in this framework. For better interpretation and understanding of the study, this chapter will also discuss the research hypotheses.

Literature Review

Oregano (*Origanum vulgare*) is a species of *Origanum* native to Europe, the Mediterranean region and Southern and Central Asia (Osabel, M. *et. al.,* 2007). In the Philippines, oregano is largely cultivated for the fresh herb market. Annual consumption of about 1.6 tons was recorded based on purchases of several food service establishments in Metro Manila (Hernandez, 2008). Like most herbs, there are two sides of oregano: the culinary and medicinal. Oregano is widely used as a condiment and its flavor and smell are greatly appreciated throughout the world for culinary applications (Tarhan *et al.*, 2007). According to Rocha-Guzma *et al.,* (2007), traditionally oregano has been consumed as a spice. In the Philippines, oregano is not commonly used for cooking but rather considered as a primary medicinal plant, useful for relieving children's cough.

For centuries people have used plants and herbs of all sorts for their apparent health giving qualities. Many herbs ingested with ordinary meals are known to include some antioxidant components. Oregano (*Origanum vulgare L.*) is one such herb, which is known to include many effective antioxidants such as rosmarinic acid, caffeic acid, and various flavonoids. According to Singletary in 2010, other bioactive compounds identified in oregano leaf include phenolic acids (caffeic acid, p-coumaric acid), rosmarinic acid and caffeoyl derivatives, ursolic acid, and carnosic acid, as well as a mixture of flavonoids. In fact, rosmarinic acid is one of the most abundant phenolic compounds present in aqueous extracts of oregano leaf. Related reports on *Origanum vulgare* have associated phenolic compound content with antioxidant activity. According to Gurpreet *et al.,* (2013), Oregano was found to be rich in crude fibre (17.43%), total phenol content (87.80 GAE/100g DW) and antioxidant activity (84.80%) which strengthens its use as a functional food.

Lemongrass is a perennial grass plant widely distributed worldwide and most especially in tropical and subtropical countries (Francisco *et al.*, 2011). Traditionally, tea made from lemongrass leaves is popular among countries of South America, Asia and West Africa. It has been widely utilized as antiseptic, antifever, antidyspeptic, carminative and anti-inflammatory effects. Shah *et al.,* in 2011 identified terpenes, alcohols, ketones, aldehyde and esters as main compounds in *Cymbopogon citratus*. Asaolu *et al.,* in 2009,

analyzed the phytochemical constituents of the leaves of *Cymbopogon citratus* and it shows that lemongrass contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids. Each of these phytochemicals is known for various protective and therapeutic effects.

Mentha is a genus about 25 species (and many hundreds of varieties) of flowering plant in the family Lamiacea (Mint Family). Several *Mentha* species are considered industrial crops as they are a source of essential oils enriched in certain monoterpenes widely used in food, flavorings, cosmetics and pharmaceutical industries (Nikitas *et al.,* 2012). The leaf, fresh oil dried is the culinary source of mint (Cabudsan *et al.,* 2008). Preparations from *Mentha sp.* are used in tradition medicine for treatment of morbid conditions like bronchitis, nausea, flatulence, colitis, liver complaints, gum and teeth diseases. This is due to its anti-inflammatory, antiemetic, diaphoretic, antispasmodic, anticatarrhal, analgesic, antibacterial and antioxidant properties (Chaiya et al., 2013).

Tea is globally one of the most popular and lowest cost beverages, next to water. It is consumed by a wide range of age groups in all levels of society. The active ingredients of tea are of interest to functional food markets (Hicks, A. 2009). According to Tano in 2012,*"The prospects of marketing tea products in the Philippines are not that low now compared before. Filipinos especially those who travel abroad and are now into health and wellness are familiar with the benefits of drinking tea."* Herbal teas are the most favored drinks among the respondents who strongly believe that these products help in digestion and relieve stress (Dagupen et al., 2009). The consumption of ready-to-drink tea, generally called iced tea, is increasing in Western countries and often overtakes the intake of traditional hot tea. Evidences showed that tea is one of the most significant sources of polyphenols in the human diet and tea consumption and polyphenol intake in general are strongly related to a lower risk for several chronic diseases (Del Rio *et al.,* 2009). RTD herbal tea beverage has gained popularity and conquered tea markets due to increased awareness of health benefits from antioxidant polyphenolics (Kim, 2008).

H_a: All variations of oregano and lemongrass ready to drink tea are not acceptable to the consumers.

H_o: All variations of oregano and lemongrass ready to drink tea are acceptable to the consumers.

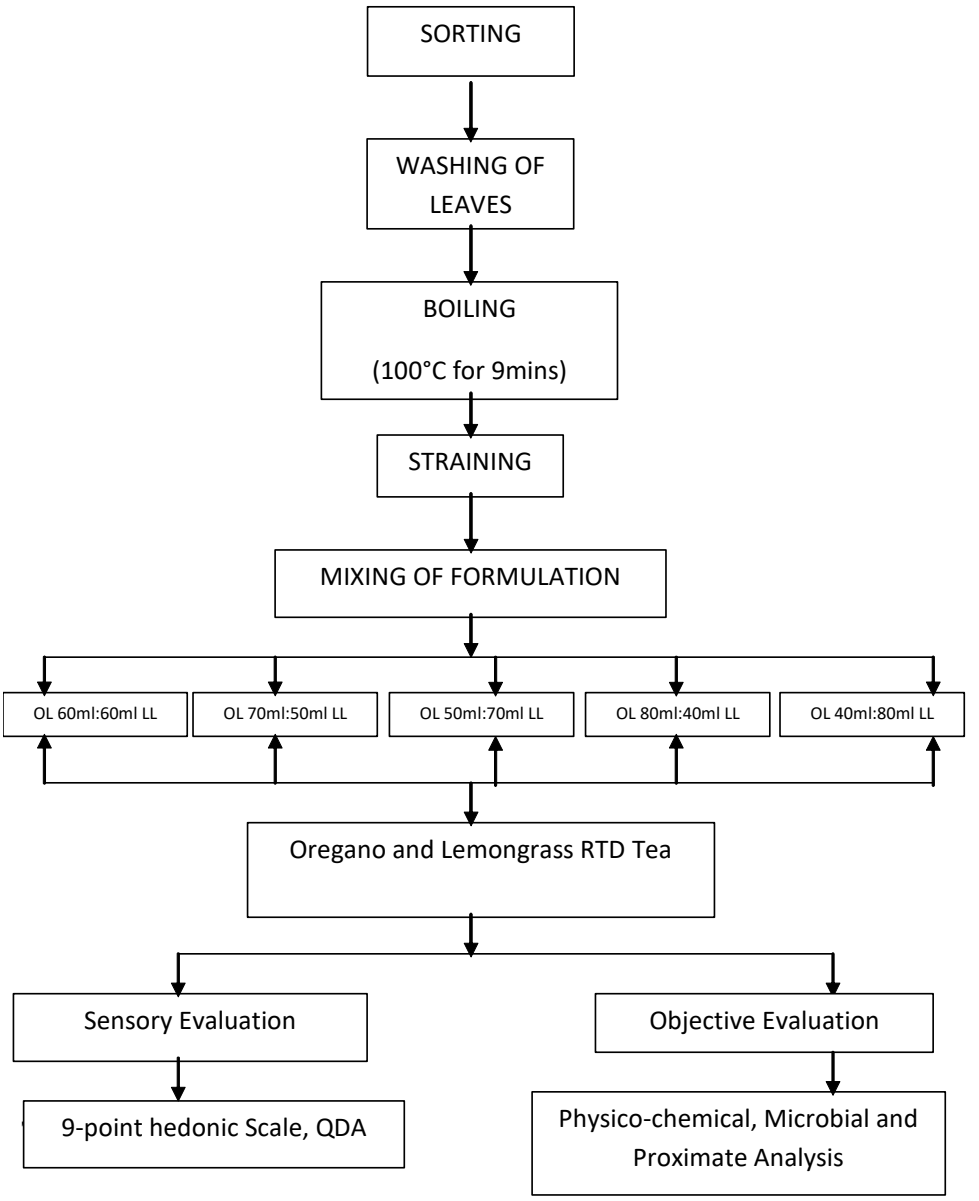

Fig. 1. Conceptual Framework of Oregano and Lemongrass Ready to drink tea

METHOD

Matured oregano leaves and mint leaves were bought in a local market in Tagaytay City. Lemongrass was bought in Polo, Valenzuela City Market. Sugar and distilled water were bought in a Supermarket. The equipment used were analytical balance, refractometer, saucepan, ladle, strainer, funnel, measuring spoon, and cups borrowed from the Food laboratory of Colegio de San Juan de Letran. The oregano, mint and lemongrass leaves were selected, trimmed and sorted. The fresh leaves were washed to remove all dirt and extraneous material while the wilted leaves were removed. 15 grams of oregano, 10 grams of lemon grass and 5 grams of mint leaves were weighed. Then it was added in 2 40ml of distilled water, boiled for 9 minutes separately. The oregano, lemon grass and mint leaves were strained using a cheese cloth to produce the extract.

From the oregano and lemon grass extract, different formulations were made with the following ratios (oregano: lemongrass): 60:60, 70:50, 80:40, 50:70 and 40:80. The 10 ml of mint extract, 5 ml of lemon essence and 50 ml of sugar solution of 35 brix solution (60 ml of water and 40 grams of refined sugar) were added to the different formulations.

Table 1. Modified Standard Formulation

Raw Materials	Formulation 1		Formulation 2		Formulation 3		Formulation 4		Formulation 5	
	Amount	%	Amount	%	Amount	%	Amount	%	Amount	%
Oregano Extract	60 ml	20.34	70 ml	23.73	80 ml	27.12	50 ml	16.95	40 ml	13.56
Lemongrass Extract	60 ml	20.34	50 ml	16.95	40 ml	13.56	70 ml	23.73	80 ml	27.12
Aqua Mint Extract	70 ml	23.73	70 ml	23.73	70 ml	23.73	70 ml	23.73	70 ml	23.73
Sugar	40 g	13.56	40 g	13.56	40 g	13.56	40 g	13.56	40 g	13.56
Water	60 ml	20.34	60 ml	20.34	60 ml	20.34	60 ml	20.34	60 ml	20.34
Lemon Essence	5 ml	1.69	5 ml	1.69	5 ml	1.69	5 ml	1.69	5 ml	1.69
Total	295 ml	100	295 ml	100	295 ml	100	295 ml	100	295 ml	100

Table 1 shows the modified standard formulations from the study of Osabel and Angeles (2007). For sensory evaluation, fifty untrained panelists evaluated the samples. The researchers used 9-point hedonic scale to evaluate the appearance, color, aroma, taste, mouth feel and general acceptability of the ready-to-drink tea. The experimentation of the study was conducted at Letran, Manila Food Laboratory. The Analysis of Variance (ANOVA) was used for the statistical analysis of the result. In occurrence of a significant difference, Duncan Multiple Range Test (DMRT) at $p<0.05$ was used, the highest amount of mean in terms of general acceptability was used for the data analysis and interpretation.

Proximate analysis (moisture, protein, fat, carbohydrates and ash) of oregano lemon grass ready to drink tea was conducted using standard procedures of AOAC (2006). Moisture content analysis was conducted using AOAC Method 925.10 (AOAC, 2006). For the analysis of protein, the Kjeldahl method of AOAC (2006) was used while AOAC Method 922.06 (AOAC, 2006) was utilized for the analysis of fat. Ash analysis was performed using AOAC Method 923.03 (AOAC, 2006). The total carbohydrates were calculated by difference:

$$\textbf{\textit{Total Carbohydrates}} = 100 - (\textit{Moisture} - \textit{Ash} - \textit{Protein} - \textit{Fat})$$

The physico-chemical analysis (vitamin C determination, total soluble solids and pH value) were done using different laboratory techniques. For vitamin C determination, it was done using High Performance Liquid Chromatography (HPLC) in SentroTek Laboratory. Total Soluble solids (TSS) were determined using a refractometer. The refractometer was standardized with distilled water. Two drops of the product were dropped on the lens of the refractometer and measured. The pH of the product was evaluated using a digital pH meter at 25°C.

Procedures for the microbiological tests were according to the Bacteriological Analytical Manual (1998) for the analyses of oregano lemongrass ready to drink tea. Total aerobic plate counts were enumerated on plate count agar (PCA) after incubation of the plates at 35°C for 48 h. Yeasts and molds were enumerated on yeast extract chloramphenicol agar

by using the surface plating technique after incubation at 25°C for 72–96 h. *E. coli* detection was performed in brilliant-green bile broth by most probable number (MPN) streak method after incubation at 35°C for 48 h. For *Staphylococcus aureus* enumeration, serial dilutions of the samples were plated on Baird-Parker agar with egg-yolk emulsion and telluride solution and incubated at 37°C for 48 h. The presence of *Salmonella* in 25 g was confirmed by pre-enrichment in buffered peptone water for 24 h at 30°C, followed by selective enrichment in Rappaport-Vasiliadis liquid medium at 43°C for 48 hours, and plating on brilliant green agar and XLD agar.

RESULTS AND DISCUSSION

Table 2 shows the result of all of the parameters for different formulations of oregano and lemongrass ready-to-drink tea. The data gathered shows that the samples A (60:60) B (70:50), C (50:70), D (80:40) and E (40:80) have no significant difference ($p > 0.05$) in terms of its color and aroma. In terms of its appearance, taste, mouthfeel and general acceptability, there is noted significant difference ($p > 0.05$) among the samples.

Based on the gathered data the mean scores for each parameters range from 6.48-7.46 which implies that all of the samples are acceptable.

Table 2. Mean Acceptability scores of Ready-to-drink Tea with variation ratio of Oregano and Lemongrass

	SAMPLE A (60:60)	SAMPLE B (70:50)	SAMPLE C (50:70)	SAMPLE D (80:40)	SAMPLE E (40:80)
APPEARANCE	7.04±1.46[a]	7.42±0.95	7.42±1.01	7.44±1.07	7.4±1.18
COLOR	7.00±1.34	7.28±0.86	7.16±1.18	7.32±1.06	7.14±1.11
AROMA	7.14±1.18	7.2±1.26	7.04±1.21	7.46±0.95	7.40±1.12
TASTE	7.36±1.14[ab]	6.48±1.46[d]	6.92±1.31[c]	7.38±0.97[a]	7.14±1.05[abc]
MOUTHFEEL	7.14±1.07[ab]	6.84±1.30[b]	6.84±1.31[b]	7.26±0.99[a]	7.08±1.12[ab]
GENERAL ACCEPTABILITY	7.04±1.24[b]	6.84±1.20[b]	7.06±1.19[b]	7.54±0.93[a]	7.16±1.30[ab]

*samples with different letters along the row showed significant difference at p>0.05

Figure 2 shows the mean of each sample in terms of its general acceptability. Based on the gathered data, Sample D (composed of 80% oregano extract and 40% lemongrass extract) is the most acceptable among the samples.

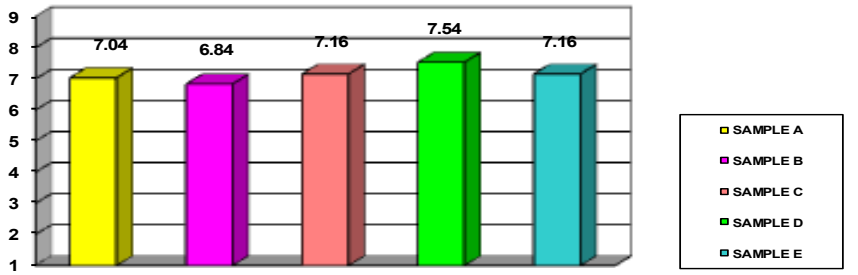

Fig. 2 Bar Graph for the General Acceptability of the product

Table 3 shows the cost analysis of product. Cost analysis of the developed product showed that it cost Php 21.85 per 500ml. This indicates that the price of oregano-lemongrass RTD tea is lower than the cost of commercially available ready-to-drink tea which is showed in table 4.

Table 3. Product Costing of Oregano lemongrass ready to drink tea beverage.

Raw Materials	Quantity	Price per serving	Percent amount used	TOTAL PRICE (Php)
Sugar	520 g	55 php/14 g	0.52 %	28.6
Distilled water	7530 ml	15. 83/ L	1. 26 %	119.22
Oregano	135g	15 php/ 40 g	0.84 %	50.4
Lemongrass	90 g	152 php/1000g	0.09 %	13.68
Mint leaves	45 g	544 php/1000g	0.045 %	24.48
Lemon essence	75 ml	900 ml /1000 ml	0.075 %	67.5
			TOTAL	303.88

Total: Php 303.88

Miscellaneous price = 10 % (Php 30.38)

= Php 30.38 + 303.88

= 334.26

Mark-up price = 15 % (Php 2.73)

= Php 334.26 +Php 2.73

= Php 336.99/ 20 servings

= Php 16.85

Price of PET bottle=Php 5.00

= Php 16.85 + Php 5.00

= Php 21.85/serving

Table 4. Cost of oregano lemongrass ready to drink tea beverage compared to commercially available product.

Product	Cost per 500ml
Oregano lemongrass RTD	21.85
Commercial RTD Tea	25.00
2nd Commercial RTD	35.00

This research work evaluates the physico-chemical properties of oregano-lemongrass ready to drink tea. The product was analyzed for its pH, ºbrix and vitamin C content. The table 4 shows the values obtained for physico-chemical properties of the product. It shows that the following parameters total soluble solids, pH and vitamin C values complied with the standards.

Table 4. Cost of oregano lemongrass ready to drink tea beverage compared to commercially available product.

Parameter	Result	Standard
pH	5.19	2.5-6.5 *(Cirigliano et al., 2000)*
°Brix	9.8 °B	9-15 °B
Vitamin C	0.01 mg/100ml	-

Proximate composition such as carbohydrates, ash, moisture, fat and protein is a chemical method of assessing and expressing the nutritional value of a food (Haque *et al.,* 2014). The results of the proximate composition of the oregano lemongrass ready-to-drink tea were shown in Table 5. The moisture content was (94.6 % w/v), which is the highest parameter, since the sample is liquid in nature.

Carbohydrates content was the second highest parameter noted having 5.4 % w/v. Carbohydrates such as glucose, sucrose, maltose and maltotriose are possible to be found widely in many foods and beverages (Ramirez *et al.*, 2004). The lowest parameter noted was the ash content (0.02%). The lower ash content in tea is due to greater moisture content in Ready to drink tea (Adnan *et al.*, 2013).

Protein and fat contents were not detected in the product. It has been reported by Oluwalana and Adedeji (2013) that progressive significant decrease in the protein and fat content may be attributed to the effect of heat process involved in the extraction which might have destroyed some amino acids with consequent reduction in total nitrogen content of the resulting beverage. Also, fat content might be attributed to the effect of direct heat on fat soluble components of the beverage during the process of extraction therefore the fat and protein content was decreased until 0 value.

Table 5. Proximate analysis of oregano-lemongrass ready to drink tea

Parameter	**Result (**%, w/v)
Total Carbohydrates	5.4
Ash	0.02
Moisture	94.6
Protein	0
Total Fat	0

Microbial analyses are important to assure that the foods are safe to consume. The analyses used are: aerobic plate count, Yeast and Molds, *Enterobacteriaceae*, *Staphylococcus aureus, Escherichia coli and Salmonella spp*. Table 6 shows the gathered results were in compliance with the microbial standards of Food and Drugs Administration (FDA) (2013) for herbal ready to drink products. Results of the microbiological tests indicate that the oregano lemongrass RTD tea was done in compliance with the Good Manufacturing Practices (GMP).

Table 6. Microbiological properties of oregano-lemongrass ready to drink tea

Parameter	Result	Standard	Reference
Aerobic Plate Count	<250 est.	10^7, maximum	FDA Summary of Current Food Standards in 2013, Herbal ready to drink products
Yeast and Molds Count	<10 est.	10^4, maximum	
Enterobacteriaceae	<10 est.	10^4, maximum	
Staphylococcus aureus	<10 est.	Negative	
Escherichia coli	<1.8	Negative	
Salmonella spp.	Absent	Negative	

Legend: <1.8 means above sample is negative for E. Coli. <10 means zero count in 10^{-1} sample dilution. <250 means count between 1 and 25 in 10^{-1} sample dilution. est.= estimated.

CONCLUSION

The researchers concluded that oregano-lemongrass ready to drink tea was acceptable to the consumers based on the gathered results of sensory evaluation. The mean scores for each parameters range from 6.48-7.46 which implies that all of the samples are acceptable. In terms of its general acceptability, sample composed of 80% oregano extract and 40% lemongrass extract is the most acceptable among the samples.

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