

Full Length Research

Effect of Rue Essential Oil on Chemical Composition, Microbiological and Organoleptic Quality of Ergo (Naturally Fermented Milk)

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Essential oils obtained from many aromatic plants have been recently gaining growing popularity and scientific interest. *Ruta graveolens* is an important medicinal and aromatic plant of citrus family. The Fresh leaves samples of Rue was harvested from Wondo Genet Research Centre subjected to essential Oil Extraction and the cow milk sample was obtained from Holeta Research Centre and the raw cow milk sample was treated with different Rue essential oil Concentration (0.5, 0.75, 1.0 and 1.5 ml / L) and control (without additive) is subjected to the Ergo or Naturally Fermented Milk Production. The Papered Ergo was analyzed for chemical, microbiological and organoleptic quality. chemical Analyze Results indicated that there was a significant difference ($P<0.05$) between the concentration, in the Moisture, fat, ash, protein, total solids (TS), Carbohydrate(lactose) content, PH and Titratable acidity (TA). Organoleptic quality Results indicated that there was a significant difference ($P<0.05$) between the concentration. From point of flavoring and antioxidant characteristics of Rue essential oils in addition to its vital role in preservation of foods, applying has met a growing interest in Dairy industrial and related sciences. Microbiological (total aerobic mesophilic bacterial, coliform, and yeast and mold counts) quality Results indicated that there was a significant difference ($P<0.05$) between the concentration, their count decreased significantly during Rue Essential oil concentration Increase, this shows the concentration have effect on microbial growth in milk during ergo preparation and it have antimicrobial properties.

Key words: Chemical, Fresh leave, Cow Milk, Ergo, Essential Oil, Fermented Milk, Microbial quality, Organoleptic quality, Rue

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INTRODUCTION

Rue (*Ruta chalepensis*) is an aromatic evergreen shrub that belongs to the family Rutaceae. It is native to the Mediterranean and is currently distributed worldwide

(Akkari et al., 2015). Analysis of the chemical composition of *R. chalepensis* extracts indicates that the leaves and stems contain alkaloids, phenols, flavonoids, amino acids,

saponins and furocoumarins, some of which are responsible for the reported activities (Kacem et al., 2015). Furthermore, rue essential oil is a valuable source of active metabolites used in different industries, including food, cosmetics, perfumes and phytotherapy. Ketone, acyclic alkenes, monoterpenes hydrocarbons, sesquiterpenes, esters and aldehydes have been identified as the main chemical groups present in the essential oil, and the ketone 2-undecanone is a characteristic compound of the Ruta species (Haddouchi et al., 2013; Ferhat et al., 2014). These compounds are produced during secondary metabolism in the plants, and their synthesis and accumulation might vary by species (Conti et al., 2013).

In Ethiopia It is called Tenadem, cultivated as ornamental and medicinal herb in gardens. Its Fresh Leave used as anti-inflammatory, antiviral and antibacterial, to protect against free radicals, to treat veins, to treated tradition of eyesight and milk flavor and Preservation. Even if their culture and attitude contributed to their usage of traditional medicine, they have no scientifically proved Amabye TG.and Shalkh TM. (2015). Ergo (Natural fermented milk) is made by natural fermentation of milk under ambient temperature, without the addition of starter cultures. This practice is technically adapted to overcome the effect of low ambient temperature, which slows down the growth of lactic acid bacteria in the absence of starter culture thus prolongs the fermentation time. The temperature and duration of incubation varies from place to place depending on the prevailing environmental conditions (Assefa et al., 2008).

This work has to be done to examine the effect of different concentration of rue essential oil on Chemical Composition, Microbiological and organoleptic quality of Ergo (Naturally Fermented Milk) instead of Rue leaf.

THE OBJECTIVES

To evaluate Effect of Rue essential oil on the Chemical Composition, Microbiological and organoleptic quality of Ergo (Naturally Fermented Milk), To evaluate effect of Rue essential oil on fermentation of Cow Milk.

MATERIALS AND METHOD

The Experiment was carried out in the Wondo Genet Natural Product Laboratory and Holeta Dairy Laboratory. A fresh leaf of Rue was collected from Wondo Agricultural Genet Research Center Gene Bank and the cow milk sample was obtained from Holeta Agricultural Research Center.

Essential Oil Extraction

The Essential oils was extracted from the fresh leaves by hydriodistillation according to Tepe et al., (2005), for 3 hrs using a Clevenger-type apparatus.

Preparation Of Ergo (Natural Fermented Milk)

The 5 liter of cow milk which obtained from Holeta Dairy Laboratory was mixed and agitated for 15 min and divided into 5 equal batches .The four batches were treated with different Rue essential oil concentration (0.5, 0.75, 1.0 and 1.5 ml / L) and one with no essential oil addition as control , and allowed for fermentation to prepare Ergo (Natural Fermented Milk) according to Assefa et al., (2008).The milk treated with Rue essential oil and control was kept for 1hr in a room temperature until used for analysis. Three replicates were prepared for each Ergo (Natural fermented milk) to determine their chemical composition, Microbiological and Organoleptic quality.

Chemical Analysis

The proximate analysis of Moisture content, acidity, fat, protein, ash, PH and TS content were done according to the method described as followed. Moisture content and Total Solid were determined by oven drying method (ILRL, 1994).Ash was determined by heating a 5g sample in a muffle furnace at 100°C for 1 hour, 200°C for 2 hours and 550°C overnight using standard method for examination of dairy products-H. Michael W and Josef F.Frank,(2004), The Protein content was determined by Titration as described by method (ILRL,1994), Fat Content was determined using Gerber method (ILRL,1994) ,The carbohydrate content was determined by difference as described by Ihekoronye and Ngoddy (1985). Titratable acidity was expressed in terms of % lactic acid this was determined by the method described by AOAC (2005). The pH was measured with a Hanna Instruments 8521 pH meter according to method Akpakpunam and Safa-Dedeh, (1995).

Microbiological Analysis

Testing for Coliform, Total bacteria, Yeast and Mold were done according to Standard Methods for the Examination of Dairy Products such as; Plate count agar was used to determine the total bacterial count (Houghtby et al., 1992). Violet Red Bile Agar (VRB) was used to determine the coliform count (Christen et al., 1992) and Acidified Potato Dextrose Agar (PDA) was used to determine yeast and mold counts (Frank et al., 1992). After the medium was prepared the medium was mixed immediately and shake for 5-10 sec. For yeast and mold, one milliliter of diluted samples was spread over pre-prepared dried plates. Then the cultured

plates were incubated at 32°C for 48 h, 37°C for 24 h and 25°C for 5 days for the total bacterial, coliform and yeast and mold, respectively (Christen et al., 1992).

Sensory Evaluation

Fifteen panelists who have experience with Ergo or Naturally Fermented Milk and regularly used its descriptive vocabulary, was participated. All the samples were evaluated for Organoleptic quality such as, flavor, sourness, color, taste, Mouthfeel and Overall acceptability using a 5-hedonic scale (1= Dislike very much, 2= Dislike slightly, 3= Neither like nor dislike, 4= like moderately, 5= like extremely) score system and the sensory profiles were conducted on coded samples (Shakerian et al., 2012).

Statistical Analysis

The data were analyzed statistically using completely randomized design within replication. The analysis of variance (ANOVA) tests were carried out by using the general linear model procedure of the statistical Analysis System Software (SAS) Version 9.. The means were separated by Duncan Multiple range test. Significant differences were determined at $p < 0.05$. The value of total bacteria, coliform and yeast and mold counts were transformed into log values.

RESULT AND DISCUSSION

Chemical Analyses

On the basis of above Result Table 1, shows that the effects of different Rue essential oil (0.5, 0.75, 1.0 and 1.5ml /L) on chemical analyses of Ergo or Natural Fermented milk ,the result was significant at ($P < 0.05$). It could be noticed that although there is revealed variations in % Moisture, % Total solid, % Ash, % protein, % Fat, % Carbohydrate, PH and % Acidity content in made by concentration of essential oil compare to each other, due to the different Essential Oil concentration affect constituent instability. The Moisture content was highest in concentration (0.1ml/L) and lowest in Concentration (1.5 ml / L). The Total Solid content was highest in concentration (1.5ml/L) and lowest in Concentration (0.1 ml /L). Ash content was highest in control sample and lowest in Concentration (1.5 ml / L).Carbohydrate (Lactose) content was highest in concentration(1.5ml/L) and lowest in Concentration (0.5 ml / L). Protein content was highest in concentration (1.5ml/L) and lowest in Concentration (0.5 ml / L). Fat content was highest in control sample and lowest in Concentration (1.5 ml / L). PH content was highest in Control sample and lowest in Concentration (1.5 ml / L). Acidity content was

highest in concentration (1.5ml/L) and lowest in Concentration (0.5, 0.75 ml / L).

Organoleptic Analyses

On the basis of above Result Table 2, shows that the effects of different Rue essential oil (0.5, 0.75, 1.0 and 1.5ml /L) on Organoleptically quality of Ergo or Natural Fermented milk, the result was significant at ($p < 0.05$). On Organoleptic quality the Sensory attributes were; Flavor, sourness, Color, Taste, Mouthfeel and Overall acceptability. The Flavor of Ergo samples containing of Rue Essential oil 0.5ml/L was generally disliked and had significantly lower acceptability scores compared to rue essential oil 1.5ml/L which gain higher acceptability than the other Result. Sourness of the Ergo which treated with Rue Essential oil 0.75ml/L was generally disliked and had significantly lower acceptability scores compared to Rue essential oil: -0.5,0.1, 1.5 (ml/L) . Taste of the Ergo which treated with Rue Essential oil 0.5ml/L was generally disliked and had significantly lower acceptability scores compared to Rue essential oil 1.5 (ml/L). It is important to note that taste may also influenced correlate with flavor. Therefore, enhancing flavor may also improve taste acceptability. Color of the Ergo which treated with all Rue Essential oil (0.5, 0.75, 1.0 and 1.5ml /L) was generally had significantly higher acceptability scores at ($p < 0.05$). Mouthfeel of the Ergo which treated with all Rue Essential oil (0.5, 0.75, 1.0 and 1.5ml /L) had higher acceptability scores was generally had no significantly at ($p < 0.05$). Therefore, enhancing flavor may also improve taste acceptability. The overall acceptability of Ergo which treated with all Rue Essential oil significantly different ($p < 0.05$). The Ergo which treated with all Rue Essential oil 1.5ml/L was obtained highest acceptability then other. The results obtained are similar to those obtained for all the other sensory attributes rated. It is reported that the acceptability of Ergo which treated with all Rue Essential oil from the above methods were influenced by Concentration of Rue Essential oil. This shows that further studies will be needed to enhancing these attributes.

Microbiological Analysis

On the basis of above Result Table 3, shows that the effects of different Rue essential oil (0.5, 0.75, 1.0 and 1.5ml /L) Ergo or Natural Fermented milk of microbiological analysis (Coliform Bacteria, Total Aerobic Mesophilic Bacteria and Yeast and Mold) the result were significant at ($p < 0.05$). The highest standard plate count was in Control sample. Minute standard plate count was confirmed in Concentration (1.5ml/L). Standard plate count for other concentration (0.5, 0.75, 0.1ml/L) varied to some extent in between the values of Control sample and

Table 1. Effect of Rue essential oil on chemical properties of Ergo or Naturally fermented milk

Concentration (ml / L)	Moisture (%)	TS (%)	Ash (%)	Protein (%)	Fat (%)	Lactose (%)	PH	Acidity (%)
0	88.10 ^c	11.10 ^d	0.52 ^a	3.98 ^b	4.50 ^b	2.89 ^c	4.27 ^b	0.71 ^b
0.5	88.77 ^b	11.23 ^c	0.50 ^a	3.92 ^c	4.50 ^b	2.31 ^d	4.32 ^{ab}	0.63 ^c
0.75	88.17 ^c	12.50 ^b	0.46 ^b	3.76 ^d	4.30 ^d	3.92 ^b	4.31 ^{ab}	0.68 ^c
0.1	90.05 ^a	9.66 ^e	0.46 ^b	3.46 ^e	4.40 ^c	1.74 ^e	4.28 ^{ab}	0.70 ^b
1.5	86.04 ^d	13.96 ^a	0.36 ^c	4.96 ^a	4.60 ^a	3.98 ^a	4.35 ^a	0.76 ^a
Lsd @ p<0.05	0.47	0.0182	0.02	0.02	0.02	0.02	0.02	0.02

Means within same column with the same letters are not significantly different (p < 0.05).

Table 2. Effect of Rue essential oil on Organoleptic quality of Ergo or Naturally fermented milk

Concentration (ml / L)	Panelist Perception					
	Flavor	Sourness	Taste	Color	Mouth feel	Over all acceptability
0	3.38 ^c	3.51 ^a	3.73 ^a	3.93 ^a	3.33 ^a	3.67 ^b
0.50	2.91 ^d	3.49 ^a	3.16 ^c	3.80 ^a	3.62 ^a	3.24 ^c
0.75	3.51 ^{bc}	2.60 ^b	3.34 ^{bc}	3.82 ^a	3.27 ^a	3.22 ^c
0.10	3.67 ^{ab}	3.47 ^a	3.53 ^{ba}	4.05 ^a	3.27 ^a	3.67 ^b
1.50	3.84 ^a	3.82 ^a	3.82 ^a	3.95 ^a	3.40 ^a	4.13 ^a
Lsd @ p<0.05	0.26	0.47	0.37	0.39	0.43	0.35

Means within same column with the same letters are not significantly different (p < 0.05).

Table 3. Effect of Rue essential oil on Microbiological quality of Ergo or Naturally fermented milk

Concentration (ml / L)	Coliform bacteria (log cfu/ml)	Aerobic Mesophilic Bacteria (log cfu/ml)	Yeast and mold (log cfu/ml)
0	4.20 ^a	7.28 ^a	6.23 ^a
0.50	4.08 ^b	7.23 ^b	6.20 ^b
0.75	3.78 ^c	7.11 ^c	6.08 ^c
0.10	3.72 ^d	6.97 ^d	5.72 ^d
1.50	3.65 ^e	6.71 ^e	5.47 ^e
Lsd @ p<0.05	0.02	0.02	0.02

Means within same column with the same letters are not significantly different (p < 0.05).

Concentration (1.5ml/L). It can be seen clearly that Microbial count decreased when the Rue Essential Oil Increased, this means it has ability to slow formation process of milk and extent shelf life of milk.

CONCLUSION AND RECOMMENDATION

On the basis of above discussion it can be concluded that

the 1.5ml/L concentration of Rue essential oil would be recommended for ergo because from other Rue concentration used it required to be effective in ergo quality, regarding in the Chemical, Organoleptical and Microbial quality. A number of options can be considered to overcome this problem, such as to view the essential oil not only as a preservative but also as a flavor component. Alternatively, it could be incorporated into products which

already have a strong flavor, or to use the most active components instead of the whole oil. This would hopefully reduce the changes in the Organoleptical properties, whilst retaining antimicrobial activity. Although Using Rue Essential oil is not popular in Ethiopia, they use only in the form of leaf without knowing its doze, this research work was conceived to establish the best effect of Rue Essential oil concentration in Ergo Production for consumer acceptance from view point of chemical, Organoleptical and Microbial quality. This research can contribute to the future scale-up of best Rue Essential Oil Concentration use instead of Rue Leaf which is not known on all over the world except Ethiopia and which is not applicable using leaf easily as essential oil in food industry

Therefore, further research work is recommended on this area due to there is no as such work is done concerning rue essential oil application on food to improve the sensory, shelf life and nutrient quality.

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