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Antioxidant Activity and Preservative Effect of Thyme (*Thymus schimperi R.*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAE designed and supervised the study; author GAH managed the literature search, performed the analyses, conducted the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To evaluate antioxidant activity and preservative effects of Thyme (*Thymus Schimperi R.*) on soybean oil, butter and meat food products.
Study Design: Complex factorial design.
Place and duration of study: Addis Ababa and Bahir Dar University, Ethiopia; between May 2010 to June 2012.
Methodology: Both leave and flower part of thyme was manually harvested, dried, milled, and sieved by 420-500µm mesh size. About 10g of prepared thyme was extracted and filtered using 125 mm diameter filter paper and stored at -18℃ till its antioxidant capacity and preservative effect of thyme was determined. Antioxidant activity of thyme crude extract was evaluated at 0.1% concentration as compared to 0.05% α-tocopherol and none-thyme extract treated soybean oil and butter samples using Rancimat and Schaal Oven test methods. The preservative effect of thyme was also studied by performing chemical and microbiological analysis on 0.1 and 0.2% thyme extract supplemented soybean oil, butter and meat for three consecutive weeks.
Results: Thyme extract increases the induction time of soybean oil from 1.92 to 3.25 hrs and five to six days as determined by Rancimat and Schaal Oven test; respectively. It also improves the induction time of butter to 5.28 hrs from 3.78hrs of control butter

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sample. Highest microbial load was obtained in controlled samples of butter and meat. Samples containing 0.2% thyme extract have lower value for all total viable microbes, mold & yeast and *enterobacteriaceae* counts of meat and butter.
Conclusion: Thyme extract significantly (P<0.05) improved both the microbial and oxidative stability of food samples. Thus, *Thymus schimperi* is a potential herb having antioxidant activity and preservative effect as it was evaluated on soybean oil, butter and meat. Thyme, which is abundantly available in Ethiopia, can be used as a source of antioxidant for production of shelf-stable food products via advance research and development activities.

Keywords: Thymus schimperi; thyme extract; antioxidant activity; induction time; preservative effect.

1. INTRODUCTION

Thyme is largely distributed in temperate zones and is uncommon in the African tropics. Ethiopia has considerably abundant Lamiaceae family herb growing at different regions and possesses a variety of the wild growing species of this family. Many species belonging to different genera of the family Lamiaceae have been reported to found in different parts of the country. The two species, *T. schimperi Ronniger* and *T. serrulatus Hochst.ex Benth*, both locally known as *Tosign,* are the endemic species represented in Ethiopia while *T. vulgaris* is a species, native to Southern Europe [1]. *Thymus schimperi* is wild growing species of thyme and comparatively well-known in Central, Eastern and Northern Ethiopia. *Thymus serrulatus* is growing in Tigray, and Bale, Showa, Gonder and Wollo are the major growing areas of thyme in Ethiopia. Wild thyme of *T. Schimperi* is harvested and dried by people living close to the town of Dinsho and near Menz (North Showa), put in plastic bags and sold to travelers on buses [2].

The main uses of thyme in culinary and food processing are defined by the properties of thyme components for aroma and flavour, antioxidant and antimicrobial activities. The thymol and carvacrol, present in thyme essence, as well as the flavonoids and other polyphenols are considered to be involved in the antioxidant activity. Rosmarinic acid, hydroxycinnamic derivatives and flavonoid compounds showed important *in vitro* antioxidant activity by inhibiting iron-induced superoxide anion formation and lipid peroxidation in microsomal and mitochondrial systems. Furthermore, the thymol present in the essential oil showed *in vitro* antioxidant activity by neutralizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical [3].

Thymus is an aromatic plant belonging to the Lamiaceae family, used for medicinal and spice purposes almost everywhere in the world. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Ethanol extract or essential oil of thyme has a significant rate of antifungal and antimicrobial activities with strongly inhibited lipid peroxidation and high-OH radical scavenging [4].

The major phenolic components in thyme extracts, especially thymol and carvacrol, present higher antioxidant activity than the well-known BHT (butylated hydroxytoluene) and α-tocopherol antioxidants [5].

The antioxidative property of thyme is important in both the medicinal and non-medicinal context. Several papers show that the essential oils and extracts of thyme exhibit antioxidative property. They are also known to inhibit lipid peroxidation. *Thymus schimperi* is rich in medicinally important constituents, thymol and carvacrol. It was found that essential oil obtained from *Thymus Schimperi* grown in Ethiopia, was rich in carvacrol (66.2%) and thymol (50%) which is responsible constituents of thyme for its antioxidant activity [6].

Antioxidants in food are capable of delaying, retarding or preventing the development of rancidity in food or other flavour deterioration due to oxidation. Antioxidants delay the development of off-flavours by extending the induction time. Addition of antioxidants after the end of this period tends to be ineffective in retarding rancidity development. The induction time is very sensitive to small concentrations of components that shorten it; the pro-oxidants, or lengthen; antioxidants. Metal ions are the most important pro-oxidants in foods, whereas antioxidants include compounds that act by radical scavenging, metal chelating or other mechanisms [7].

Some plant extracts have been known as antimicrobials as well as antioxidants in food systems. Thyme is the one among the potential herbs for extracting natural antioxidants. Ethiopia has abundant wild thyme (*Thymus Schimperi*) to extract natural antioxidant and antimicrobial from this potential herb. Thyme leaves are used in Ethiopia extensively as spice/ additive to flavor a wide range of food and beverage products. However, antioxidant potential of this herb was not yet well fully studied and exploited properly to improve the shelf-stability of food products. Therefore, the purpose of the research work was to evaluate the total antioxidant activity of *Thymus Schimperi* extract using lipid oxidation inhibition system and determine its preservative effect on oil, butter and meat.

2. MATERIALS AND METHODS

2.1 Thyme Collection, Transportation and Storage

Thyme (*Thymus Schimperi* R.*)* was obtained from Tarmaber which is 180km away from Addis Ababa, Ethiopia. Both leave and flower part of thyme were manually collected and dried with sun drying system in protective and shaded way. The shade dried thyme was packed in polyethylene plastic bags and taken to Bahir Dar University, Technology Institute, Food Chemistry and Analysis Laboratory for further analyses.

2.2 Setting Extraction Parameters

The independent variables studied were: extraction solvent (ethanol) concentration of (0-97%), extraction temperature (20-40℃) and extraction time (3-4hrs) for actual variable levels. For each variable, an experimental range was adjusted based on the results of literature data [8] and the performance of preliminary experiment trials. In this study, the particle size was controlled as constant since the 420-500µm is optimal for extraction, while smaller particles may become slimy during extraction and create difficulty during filtration [9].

2.3 Thyme Extract Preparation

Samples of about 10g of dried, milled, powdered and sieved thyme were extracted with 100mL of solvent. The extraction process was performed using a magnetic stirrer with heat plate. After extraction, the samples were filtered using 125 mm diameter filter paper. The

solvent ethanol was separated from extracts using a rotary evaporator (Buchi Rota-vapor R-124, Switzerland) under vacuum at 45℃ and then weighed to measure thyme extraction yield. The concentrated thyme extract was stored at -18℃ till its antioxidant capacity was determined. Whereas, aqueous extract of thyme was further freeze dried for antioxidant activity evaluation.

2.4 Evaluation of Thyme Antioxidant Activity

The antioxidant activity of thyme extract was determined by Rancimat (Model 743, Metrohm, Switzerland) and Schaal Oven test method to get induction time; taking soybean oil and butter as a real food model system (substrate) for lipid oxidation analysis occurs in lipid foods. The induction time for the formation of oxidative products of oxidizing substrate were measured and converted to protection factor for antioxidant activity evaluation.

In the case of natural antioxidants, higher concentrations (0.05-0.2%) are necessary because of their lower activities and presumed lower toxicity. The concentration of 0.1% was studied as it is most often used in the research as a model substance representing natural antioxidant [10]. Samples of thyme extracts were added to about 5.0g refined soybean oil and butter at concentration of 0.1% (w/w). For comparison, vitamin E (α- tocopherol) was added to the oil and butter at 0.05% (w/w) concentration. At the same time, soybean oil and butter samples without thyme extract were prepared as negative control to calculate the protection factor.

Three parallel treatments are filled into the reaction vessels and introduced in the heating blocks. The treatments were kept at stable temperature of 130℃ and continuous air stream of 20L/hr pumped through the samples. The induction time was detected and recorded by computer fitted to the Rancimat. Antioxidant activity of thyme extract was expressed as a protection factor. The protection factor (PF) was calculated as:

$$PF = \frac{IT_S}{IT_O}$$

According to the method described by [11], Antioxidant activity of thyme extract was calculated by measuring induction time as independent variable.

$$\mathrm{AA_t} = \frac{[\mathrm{PF} - 1]}{[\mathrm{AH}]}$$

Where: ITs = The induction time of the sample (oil/butter with thyme extract) [hr]
ITo = The induction time of control soybean oil [hr]
AA_t = Antioxidant activity of thyme extract
[AH] = Concentration of thyme extract added to the oil or butter

Based on the calculation result, the protection factor can be interpreted in three ways:

PF=1 or if ITs = ITo, the thyme extract does not have antioxidant activity
PF<1, the thyme extract shows pro-oxidant activity
PF>1, the thyme extract shows antioxidant activity

In the Schaal oven test, about 40g of samples of refined soybean oil supplemented by 0.1% thyme extract were put in 50ml bottle and placed in a drying oven at 60℃. For comparison, both positive (α-Tocopherol at 0.05%) and negative (without thyme extract) treatments were prepared and stored in the same condition. For each treatment, the time required to reach at the targeted peroxide value of 20 $mEqO_2$/kg soybean oil (the point at which soybean oil become rancid and has poor quality) has been taken as induction time to evaluate thyme antioxidant activity. The peroxide value was determined based on [12] using official method 965.33.

2.5 Preservative Effect of Thyme

Thyme extract was added to each test samples of meat, butter and oil at three different concentration levels; 0, 0.1and 0.2% and the samples were stored for 7, 14 and 22 days. A total of eighteen butter samples with 40g weight, were separately stored at 4℃ refrigeration temperature for microbial and chemical analysis. For each analysis three butter samples were treated as blank (without thyme extract), the other six samples were prepared with 0.1% and 0.2% crude thyme extract.

For microbiological analysis of meat, three treatments were prepared with 0, 0.1 and 0.2% crude thyme extract. Each treatment has about 100g of meat and stored at 4℃ refrigeration temperature. In every week, about 25g sample was taken from each treatment for analysis of total aerobic viable count [13], mold and yeast and pathogenic microbial count [14,15].

Preservative effect of thyme crude extract was also studied on soybean oil and nine soybean oil samples were prepared. Each has a weight of about 40g and treated with 0, 0.1 and 0.2% crude thyme extract. The samples were stored at room temperature in dark place for three consecutive weeks. The free fatty acid value of butter and soybean oil was evaluated according to [12] official method number 940.28 for each treated samples per each storage week.

2.6 Experimental Design and Data Analysis

A complex factorial design (a design which considers three independent variables (2^3) simultaneously) was used to study the effect of extraction parameters on thyme antioxidant activity. Data obtained from the experiment were analyzed using Analysis of Variance (One way ANOVA) method to compare the mean value and standard deviation of each treatments at significant level of $p<0.05$ by JMP statistical analysis software version 5.0 and Design Expert Software Version 7.0.0.

3. RESULTS AND DISCUSSION

3.1 Effect of Extraction Parameters on Thyme Antioxidant Activity and its Extract Yield

The results presented in Table 1 showed that the concentration of extraction solvent had significant effect ($p<0.05$) on thyme antioxidant activity. It was noted that the ethanol concentration had critical role in the extraction of soluble components from different natural products [16]. Thyme antioxidant activity determination with Rancimat method shows that thyme crude extract obtained by distilled water resulted in higher antioxidant activity.

Extraction temperature was found to be the most significant factor affecting antioxidant activity of thyme at $p<0.05$ level. Induction time was decreased with increased thyme extraction temperature. Based on the obtained result, antioxidant activity of thyme crude extract was increased proportionally with the decreasing of extraction temperature, reaching maximum values at room temperature. The loss in antioxidant capacities of plant extracts at high extraction temperature was likely due to degradation of phenolic compounds which were mobilized at low temperature [17].

Table 1. Effect of extraction parameters on thyme antioxidant activity

S. No.	Extraction parameters			Induction time (hr)	Protection factor $PF = \frac{IT_S}{IT_O}$	Antioxidant activity $AA_t = \frac{[F-1]}{[AH]}$
	Ethanol Conc. (%)	Temp.(℃)	Time(hr)			
1	97	40	4.0	2.65±0.08^{d}	1.38±0.041^{d}	3.8±0.410^{d}
2	0	20	3.0	3.92±0.25^{a}	2.04±0.130^{a}	10.4±1.40^{a}
3	0	40	3.0	3.22±0.04^{b}	1.68±0.021^{b}	6.8±0.21^{b}
4	0	40	4.0	3.09±0.18^{bc}	1.61±0.093^{bc}	6.1±0.92^{bc}
5	0	20	4.0	4.09±0.39^{a}	2.24±0.203^{a}	12.4±2.03^{a}
6	97	20	4.0	2.87±0.09^{cd}	1.50±0.047^{cd}	5.0±0.47^{cd}
7	97	20	3.0	2.62±0.16^{d}	1.40±0.085^{d}	4.0±0.85^{d}
8	97	40	3.0	2.86±0.22^{cd}	1.49±0.120^{cd}	4.9±1.20^{cd}
9	48.5	30	3.5	3.04±0.10^{bc}	1.58±0.052^{bc}	5.8±0.52^{bc}

Means within the same column followed by the same letters are not significant difference at $p<0.05$

All values are mean ± standard deviation. Where: IT_s, is Induction Time of thyme antioxidant, IT_0, Induction Time of control and AA_t, is antioxidant activity of thyme

In general, the maximum thyme antioxidant activity was achieved at extraction time of 3.5 hour. After this point, thyme antioxidant capacity was decreased. It was believed that prolonged extraction time would lead to exposure of more oxygen and thus increase the chances for occurrence of oxidation on phenolic compounds [18].

The result of one-way ANOVA showed that the distilled water extract exhibited significantly higher total antioxidant activity ($p<0.05$) than that of ethanol thyme crude extract. It could be concluded that the different polarity of the extracts might contain different antioxidant constituents that demonstrated a varying reactivity in the model food substrates.

3.2 Evaluation of Thyme Antioxidant Activity

3.2.1 Rancimat method

Table 2 contains thyme antioxidant activity of three samples treatments of soybean oil and butter performed by rancimat method. Induction time was automatically determined by computer connected to Rancimat equipment for each treatment. Thyme extract shows an antioxidant activity ($p<0.05$) in both oil and butter since the protection factor of thyme extract treatment is greater than one.

Table 2. Antioxidant activity of thyme evaluated by Rancimat on soybean oil and butter

Model substrates	Treatments	Induction time (hr)	Protection factor	Antioxidant activity
Soybean oil	Oil with thyme extract	3.25±0.02[b]	1.69±0.010[b]	6.9±0.10[b]
	Oil with α-tocopherol	4.98±0.10[a]	2.59±0.052[a]	15.9±0.50[a]
	Oil alone /Control/	1.92±0.08[c]	1.00±0.042[c]	ND
Butter (Lame Dairy PLC)	Butter with thyme extract	5.28±0.08[c]	1.40±0.021[c]	4.00±0.21[c]
	Butter with α-tocopherol	7.12±0.06[a]	1.88±0.015[a]	8.80±0.30[a]
	Butter alone /Control/	3.78±0.10[e]	1.00±0.026[e]	ND

Means within the same column followed by the same letters are not significant difference at p<0.05, All values are mean ± standard deviation.

Where: ND- Antioxidant activity of control was not detected for soybean oil and butter samples without thyme extract

The higher induction period of the soybean oil and butter with the thyme extract added, compared to the control implies the better the antioxidant activity of thyme. Thyme antioxidant was effective in maintaining stability of the oil and butter for extended time when treated samples were exposed to accelerated condition in Rancimat. But, thyme extract has low antioxidant activity compared to vitamin E (α-tocopherol) applied at 0.05% concentration as positive treatment.

3.2.2 Evaluation of thyme antioxidant activity in butter

Data of Induction time and protection factor were presented in Table 3 for butter samples collected from different locations. These butter samples were collected from Tarmaber, Sheno, BahirDar, Hirut Dairy PLC and Lame Dairy PLC. All butter samples were treated under the same way during transportation, storage and evaluation. This data table do not have antioxidant activity column since all samples were determined without addition of thyme extract. In this case, the study was intended examine thyme feed cows can provide butter enriched in antioxidants and see the selling price of these butter in the market. On market, some butter have higher selling price. This study wants to consider the perception regarding to the antioxidant content of the butter.

The cumulative antioxidant activity of thyme was also evaluated in butter, collected from Tarmaber, Sheno, BahirDar, Hirut dairy PLC and Lame dairy PLC to examine the quality of butter related to its antioxidant content.

Table 3. Antioxidant activity of butters collected from different locations as determined by Rancimat

Locations of butter samples taken	Induction time (hrs)	Protection factor
Tarmaber	4.66±0.08[a]	1.12±0.020[a]
Sheno	3.78±0.14[b]	0.96±0.035[b]
Hirut Dairy PLC	4.22±0.37[ab]	1.07±0.043[ab]
Lame Dairy PLC	3.96±0.11[b]	1.00±0.028[b]
BahirDar	2.24±0.28[c]	0.57±0.071[c]

Means within the same column followed by the same letters are not significant difference at p<0.05, All values are mean ± standard deviation.

The results presented in Table 3 shows; these butter samples collected from different locations have different antioxidant content. Both Tarmaber and Hirut Dairy PLC butters exhibit higher antioxidant activity since they were obtained from cows eating thyme together with their feeds. Butter of Tarmaber has higher induction time and protection values (4.66±0.08 hrs and 1.12±0.02; respectively) than butter of other locations and implies better antioxidant activity. It also has unique yellowish color due to green thyme used as cow's feed. This in turn indicates that butters enriched by caroteniods and can have antioxidant properties. Whereas, butters taken from Sheno and BahirDar locations did not have antioxidant activity as compared to that of Tarmaber and Hirut dairy PLC butters as indicated in Table 3. Regularly, butter sellers give higher price for Sheno butter and sell it to customers by advocating the presence of thyme in the butter. However, based on this study, Sheno butter was not as such quality butter enriched by antioxidant as compared to Tarmaber and Hirut dairy PLC butters which have higher antioxidant content. It was suspected that in Sheno area might be sold butter arrived from other localities to share the market channel with higher prices by misbranded as Sheno butter. Other localities butter quality doesn't much with butter obtained from thyme growing areas where cow's feed thyme. Further traceability research study can be performed to give concrete explanation on butter sources sold at Sheno. The report [19] stated that the antioxidant and preservative effect of thyme can be improved through animal feed ration preparation. Some private companies and dairy farmers are more focused on thyme-based feed production to manufacture high quality butter which might be exported to gulf countries in the near future.

3.2.3 Schaal oven test method

The oxidative rancidity index employed to analyze the antioxidant activity of thyme was the peroxide value. In this case, the induction time is taken as storage time which required for each treatment to reach the peroxide value of 20mEqO_2/kg soybean oil where the oil has poor quality and assumed to be rancid. From Table 4 it can be seen that the effect of thyme extract addition on the peroxide value of soybean oil stored at 60℃.

As overall observation, the addition of thyme antioxidant retarded the formation of peroxide value of soybean oil compared to the control. Initially, the peroxide value the oil was 4.00 mEq oxygen/kg of soybean oil. From the results obtained, the peroxide value increased with storage time. The peroxide value of blank treatment significantly increased from 4.00±0.00 to 58.33±0.71 within seven day of storage time. Thyme extract containing oil samples have lower peroxide value than untreated blank samples (control).

Table 4. Peroxide value for evaluating antioxidant activity of thyme using Schaal Oven test

Storage time (day)	Treatments		
	Control	(0.1%) thyme extract	(0.05%) Vitamin E (α-Tocopherol)
0	4.00±0.00^{e}	4.25±0.35^{f}	4.00±0.00^{f}
2	8.50±0.71^{d}	7.00±0.00^{e}	6.40±0.56^{e}
4	11.00±0.82^{d}	8.10±0.71^{d}	7.60±0.56^{d}
5	19.67±0.47^{c}	13.50±0.71^{c}	12.25±0.35^{c}
6	30.30±0.80^{b}	18.33±0.71^{b}	16.40±0.56^{b}
7	58.33±0.71^{a}	28.70±0.35^{a}	19.86±0.42^{a}

All values are mean ± standard deviation.

Means within the same column of each treatment followed by the same letters are not significant difference at p > 0.05.

Induction time required for reaching the targeted peroxide value of control and thyme treatments were found to be five and six days respectively. Whereas, reference antioxidant (α-Tocopherol), needs seven days. Thyme showed significantly higher antioxidant activity (p<0.05) than the negative control. Reference antioxidant exhibited stronger antioxidant activity (p<0.05) than both the control and thyme treatments. In this case, α-Tocopherol was found to be the most effective antioxidant since there was a slightly increment in peroxide value under the seven days storage time. Schaal oven test method of thyme antioxidant activity determination was not well comparable with Rancimat method. This behavior can be attributed due to the very low solubility of thyme extract in the oil. It has been previously found that antioxidant's protecting efficacy increased when the continuous airflow facilitates emulsification [20].

3.3 Preservative Effect of Thyme

3.3.1 Chemical analysis

3.3.1.1 Free fatty acids

As mentioned in Table 5, FFA value of blank treatment of soybean oil (oil without thyme extract) was found to be 0.296±0.02. After one week, the FFA value was promoted to 0.340±0.002. At the completion of three week storage time, FFA value of the control treatment of soybean oil was increased to 0.423±0.02. This change of FFA content of was significant (p<0.05) according to statistical analysis. While, 0.1% thyme extract treated soybean oil, have the free fatty acid value of 0.231±0.01 and 0.353±0.02 at the first and third week analysis respectively. Whereas, 0.2% treated refined soybean oil have the value of 0.198±0.04 and 0.284±0.21 during the 1st and 3rd week storage time.

Table 5. Free fatty acid value of soybean oil and butter treated by thyme crude extract

Food substrates	Storage weeks	Concentration of thyme crude extract (%)		
		0	0.1	0.2
Refined soybean oil	1st	0.296±0.02[a]	0.231±0.006[ab]	0.198±0.041[b]
	2nd	0.340±0.002[a]	0.296±0.027[b]	0.256±0.035[c]
	3rd	0.423±0.02[a]	0.353±0.021[b]	0.284±0.21[b]
Butter (Lame Dairy PLC)	1st	0.353±0.02[a]	0.31±0.04[a]	0.296±0.02[a]
	2nd	0.423±0.04[a]	0.381±0.02[a]	0.377±0.01[a]
	3rd	0.592±0.03[a]	0.458±0.01[b]	0.403±0.01[b]

Means within the same row of food substrate and column of thyme extract concentration followed by the same letters are not significant difference at p<0.05
All values are mean ± standard deviation.

In the case of butter, at the 1st and 3rd week of storage time, the free fatty acids values of control treatment were 0.353±0.02 and 0.592±0.04 respectively. The FFA value of 0.1% thyme extract treated butter was 0.31±0.04 at 1st week. After three week, its FFA value was increased to 0.453±0.01. Treatment of 0.2% thyme extract has a free fatty acid value of 0.296±0.02 and 0.403±0.01 at 1st and 3rd week of butter storage time respectively. The value of FFA, decrease with increasing thyme extract concentration across the storage week of butter. Thyme and cumin essential oils could prevent oxidation in butter stored at room temperature, and at 200ppm the essential oils were more effective than BHT in inhibiting lipid oxidation in the butter [21].

3.3.1.2 Peroxide value

The peroxide value of control treatments of soybean oil was 5.20±1.13 as shown in Table 6. It was increased to 15.0±1.41 at the end of storage time. The peroxide values of 0.1% and 0.2 % thyme extract treated oil samples were changed from 4.50±0.71 to 6.50±0.71 and 5.00±1.41 to 6.00±0.00 at the 1st and 3rd week of analysis respectively. These changes were significantly indicated the noticeable phenomenon of lipid oxidation. There were statistical differences ($p<0.05$) among control and thyme extract treatments. One-way ANOVA analysis result, showed that refined soybean oil samples treated by 0.1 and 0.2% thyme extract have significantly lower peroxide value ($p<0.05$) compared to untreated blank oil sample.

Table 6. Peroxide value of thyme extract treated with soybean oil and butter

Food substrates	Storage weeks	Concentration of thyme crude extract (%)		
		0	0.1	0.2
Refined Soybean oil	1st	5.20±0.35^{a}	4.50±0.41^{a}	5.00±0.48^{a}
	2nd	9.50±0.61^{a}	7.00±0.50^{ab}	5.50±0.42^{b}
	3rd	15.0±0.70^{a}	6.50±0.71^{b}	6.00±0.05^{b}
Butter (Lame Dairy PLC)	1st	3.25±0.56^{a}	1.50±0.45^{a}	1.50±0.43^{a}
	2nd	15.0±0.72^{a}	10.0±0.73^{b}	9.50±0.40^{b}
	3rd	21.0±0.76^{a}	15.0±0.71^{b}	13.5±0.42^{b}

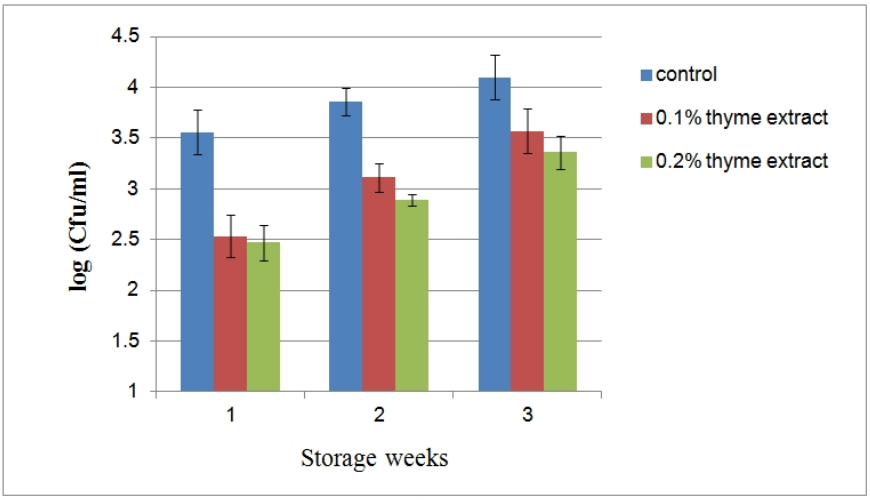
Means within the same row of food substrate and column of thyme extract concentration followed by the same letters are not significant difference at $p<0.05$, All values are mean ± standard deviation.

In butter, the control has higher peroxide values ranged from 3.25±1.06 to 21±1.41 at the 1st and 3rd week of storage time. Butter treated with 0.1% thyme extract has peroxide values of 1.5±0.71 and 15±1.41 during storage weeks. After the 2nd week of storage time, the result of one-way ANOVA analysis exhibited that thyme extract has significant effect ($p<0.05$) as compared to untreated butter sample. Both 0.1 and 0.2% thyme extract treated butter samples show lower peroxide value.

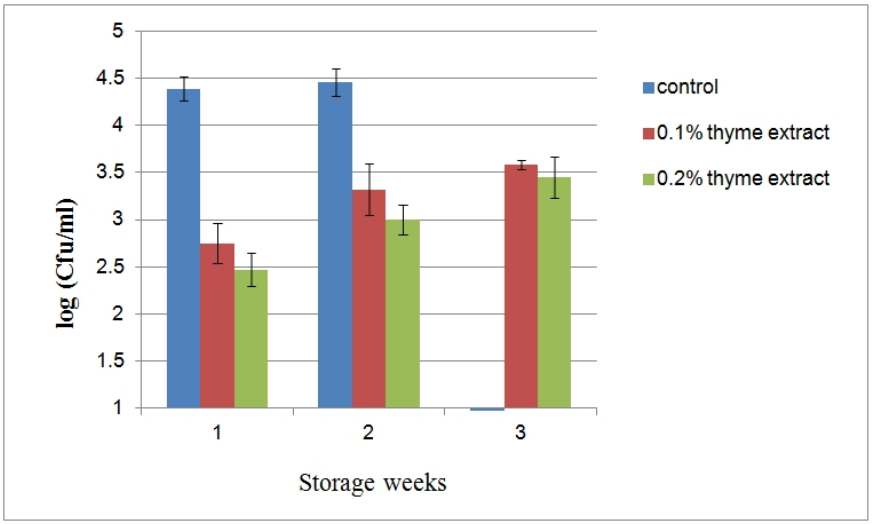
3.3.2 Microbiological analysis

3.3.2.1 Aerobic total viable count

The results of each treatment of both butter and meat were expressed by the logarithm of colony forming units obtained by direct count of colonies on each serial dilution per ml of sample inoculated to plate count agar medium in duplicate. At all three weeks of butter cold storage, control sample showed the higher colony count comparing to other samples contained thyme extract at 0.1 and 0.2% concentrations. The value of its aerobic plate count significantly increased during each cold storage week of butter. In Fig. 1(a), both 0.1 and 0.2% thyme extract treated butter samples showed lower value of aerobic plate count. It is quite clear that thyme extract has antimicrobial effect in preservation of butter.



(a)



(b)

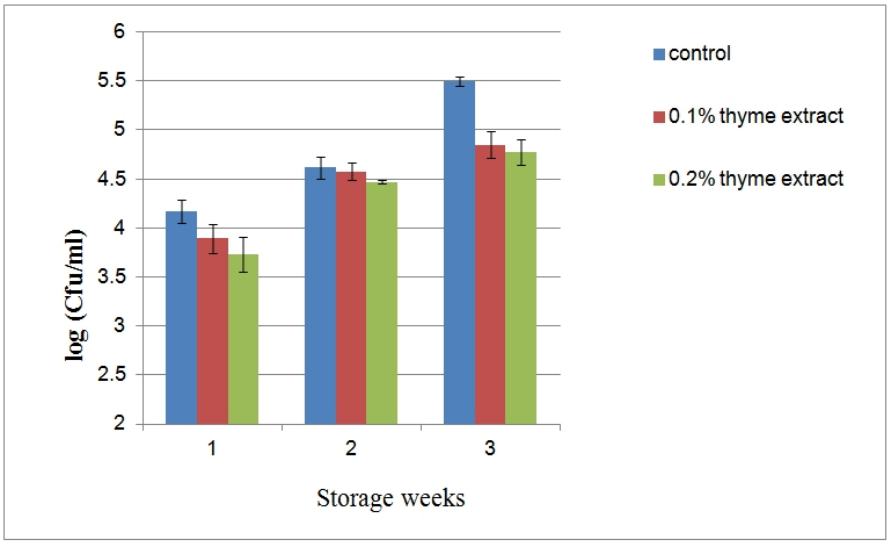
Fig. 1. Aerobic viable count (a) Butter (b) Meat

In the case of meat, the colony count values of control sample were higher than other thyme extract treated samples during the first two weeks of meat cold storage time. But at the third week of meat storage, it was very difficult to count colonies grown on plate count agar medium. In meat, both concentration of thyme crude extracts (0.1 and 0.2%) significantly decreased the value of aerobic plate count as indicated in Fig. 1(b).

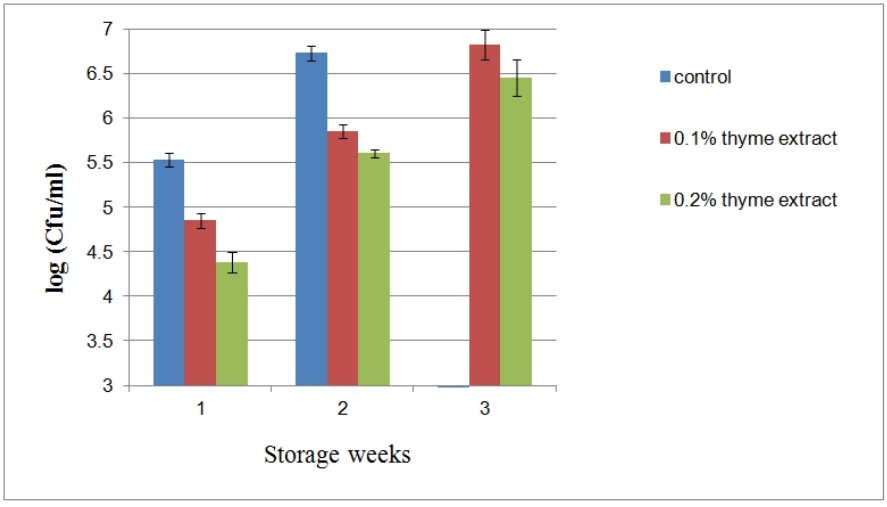
In Fig. 1(a,b), it has been clearly seen that 0.2% thyme extract has lower count value in each cold storage week of meat. The pattern of the colony count value can be arranged as: control sample > 0.1% thyme extract >0.2% thyme extract treated samples. These strong antimicrobial activities are mostly due to the presence of phenolic compounds such as thymol and carvacrol, and to hydrocarbons like γ- terpinene and p-cymene [22].

3.3.2.2 Mold and yeast count

The values of mold and yeast count for control sample of butter were higher than thyme crude extract treated samples in all three weeks of cold storage time. The values were increased significantly during each week. As shown in Fig. 2(a), 0.1 and 0.2% thyme crude extract treated butter samples showed lower value of aerobic mold and yeast count. It is quite clear that thyme extract has antimicrobial effect against the growth of both mold and yeast in cold stored butter.



(a)



(b)

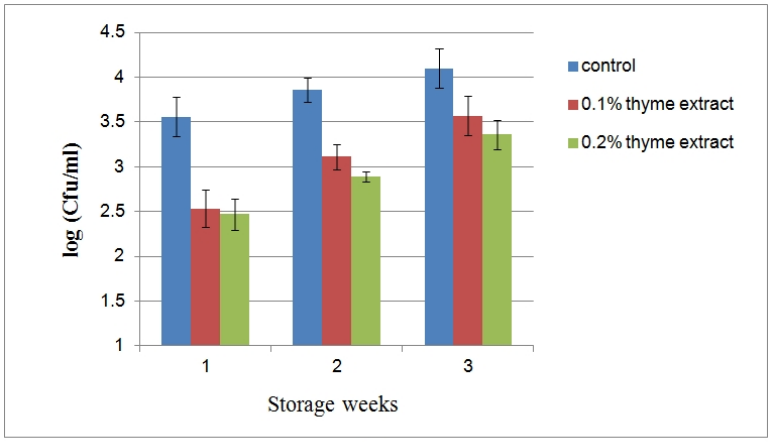
Fig. 2. Mold and Yeast Count (a) Butter (b) Meat

Higher values of aerobic mold and yeast count were obtained in the control sample of meat during the 1st and 2nd weeks of cold storage time. However, its value at the 3rd week of

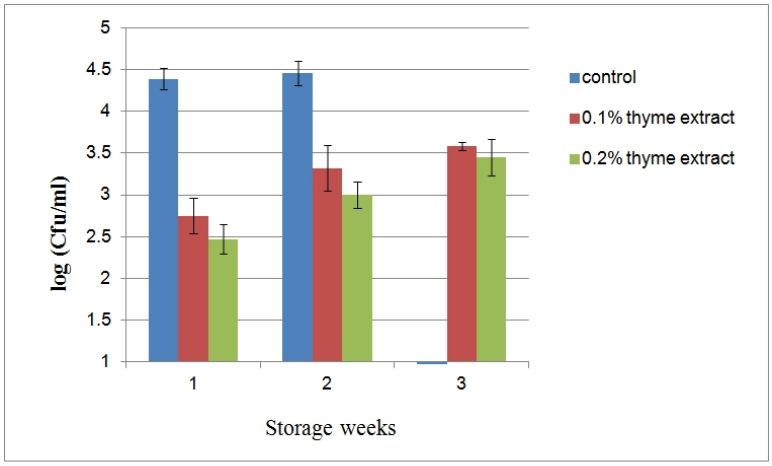
analysis was too difficult to count colonies grown on Potato Dextrose Agar medium. Both concentration of thyme crude extracts (0.1 and 0.2%) significantly decreased the value of aerobic mold and yeast growth count during the whole weeks of analysis. In Fig. 2(b), it has been also clearly seen that 0.2% thyme extract has lower count value in each cold storage week of meat. Antifungal activity of three essential oils (thyme, summer savory and clove) is evaluated in culture medium and as a real system in tomato paste (*in vitro* and *in vivo*) and the results clearly showed that *in vitro* each essential oil had notable antifungal activity [23].

3.3.2.3 Enterobacteriaceae count

As shown in Fig. 3(a), it could be observed that control sample of butter (butter alone) had the highest counts of *enterobacteriaceae* at all three weeks of cold storage compared to other treatments. The count of *enterobacteriaceae* of butter significantly increased during each weeks of cold storage at 4℃. Samples of butter treated by both 0.1 and 0.2% thyme crude extract showed lower value of *enterobacteriaceae*.



(a)



(b)

Fig. 3. *Enterobacteriaceae* count for (a) Butter and (b) Meat

The values for *enterobacteriaceae* count of control sample of meat were higher than other thyme extract treated samples during the two weeks of meat cold storage time as shown in Fig. 3(b). But, at the third week of meat storage, it was very difficult and too much to count colonies grown on plate count agar medium. Both concentration of thyme crude extracts (0.1% and 0.2%) significantly decreased the value of *enterobacteriaceae* count. For successful applications of thyme in different food systems, potential interaction between thyme extract and food components have to be determined.

4. CONCLUSION

It is evident from the result of this work that antioxidant activity of thyme crude extract was depend largely on the extraction parameters (solvent concentration, extraction temperature and extraction time), the kind of food substrate, concentration of the extract being used in the substrate and the method of choice for antioxidant activity test. Higher antioxidant activity of thyme was found by distilled water extraction at room temperature for 3.5 hours. Thyme and α-Tocopherol treated samples of soybean oil and butter revealed induction time of 3.25±0.02 and 4.98±0.10 hrs; respectively as determined by Rancimat. In Schaal Oven test method, they have six and seven day's induction time in soybean oil and butter; respectively. Thyme also has 5.28±0.08 hrs induction time when it was evaluated in butter by Rancimat method.

It can be concluded that crude thyme extract contains an effective antioxidant in stabilizing refined soybean oil and butter. The study also provides an insight into understanding the behavior of adding natural antioxidants to food products resulted in enhancement on oxidative and microbial stability. Results of thyme preservative effect revealed that treatments of 0.2% thyme extract significantly improve microbial stability of soybean oil, butter and meat food products. The values of *enterobacteriaceae* counts decreased as the concentration of thyme extract increase with the same storage week of both meat and butter. Hence, Ethiopian thyme has acceptable antioxidant activity and preservative effect as observed on thyme extract treated food products. In conclusion, thyme crude extract has the beneficial effect in controlling the microbial load of both meat and butter during three weeks of storage at 4ºC compared with control samples.

Health benefits, phytochemical composition, antioxidant potential, antimicrobial activity, efficacy of thyme extract within food products and food preservative effect to increase shelf stability of food products need further research. Persistent research activities are required in order to examine the quality of meat and butter on thyme feed animals; which is the current indigenous practice of Ethiopian highland farmers. Currently, combination of thyme with tea and traditional dishes become popular in the Ethiopian context. However, blending and fortification with other plant and animal origin agricultural produces requires investigation in order to maximize the utilization of available resource via value addition at industry level. Improvements of the existing indigenous practices and utilization aspects in African countries are vital by means of realizing the potential of agro-industries as engine for economic development.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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