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Optimizing the extraction of essential oil from cinnamon leaf (*Cinnamomum verum*) for use as a potential preservative for minced beef

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Abstract

Cinnamon leaf essential oil extraction using steam distillation method is a time-consuming and energy-intensive process. Furthermore, a lower yield and a higher rate of product degradation are this method's main drawbacks. Thus, the goal of this research is to optimize the extraction process parameters of cinnamon leaf essential oil in response to maximizing the yield while retaining quality by using response surface methodology (RSM). The application of extracted essential oil on minced beef to assess its preservative effect was also the other objective of this research. Extraction time (120–210 min), extraction temperature (105–115 ℃), and feed mass (300–600 g) were the chosen independent variables of the optimization experiment using central composite design (CCD). Furthermore, the extracted essential oil's antibacterial and microbiological preservative activity on minced beef was evaluated. At extraction time of 175.43 min, extraction temperature of 105 °C, and a feed mass of 600 g, the optimum predicted value of cinnamon leaf essential oil yield and cinnamaldehyde concentration (% area) was 2.9% and 34.6%, respectively. Moreover, the second-order polynomial equation fits the experimental data for 20-run experimental data. The chemical composition of cinnamon leaf essential oil extracted at optimal conditions was dominated by eugenol (60.68%) and cinnamaldehyde (33.94%). Additionally, the optimally extracted cinnamon essential oil inhibited the growth of bacteria, particularly gram-positive bacteria. After twenty-one days of storage at 4 °C, total viable count of minced beef seasoned with cinnamon essential oil at concentration of 1.2% (v/v) was lower than 10^6 CFU/g. To conclude, optimized cinnamon leaf essential oil extraction process provides better yield while retaining its functional properties.

Keywords Essential oil, Extraction time, Extraction temperature, Feed mass, Antibacterial, Antioxidant, Minced beef

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Introduction

Essential oils are concentrated hydrophobic liquid aromatic and volatile compounds generated from the cytoplasmic fluid of the plant and found as small droplets located at the intracellular space [1]. For the plant, the major functions of these oils are either to protect it from pests and/or predators, or to facilitate plant-to-plant communication [2]. For a variety of uses, these essential oils have been utilized for millennia. They have been utilized as food additives, perfumery scents, and active medicinal compounds. From the nearly 3000 plant species that have been studied for the composition of their essential oils up to this point, hundreds have been used commercially [2].

Cinnamon bark and leaves are frequently utilized to make essential oils for a variety of applications [3, 4]. There are over 250 species in the genus *Cinnamomum*, with *C. verum* being the most widely utilized species for the production of essential oils. Besides, the use of *C. verum* essential oil for medical and food additives is a long-standing tradition [5, 6]. Up to 124 compounds were identified from essential oil extracted from *C. verum* [7]. From these compounds, eugenol, cinnamaldehyde, and camphor are the principal compound found in the essential oils extracted from leaves, barks and root-bark cinnamon plant [5, 8, 9]. These compounds had shown in the previous studies for their good anti-oxidant, and antimicrobial effect [3, 10].

Essential oils from aromatic plants can be extracted by either conventional (hydro-distillation, steam distillation, solvent extraction etc.) or advanced (supercritical fluid extraction, microwave-assisted extraction, microwave-assisted extraction, microwave steam distillation etc.) techniques [11, 12]. Each of these extraction methods, however, has advantages and disadvantages of their own. The most well-known drawbacks of the traditional essential oil extraction method include the lengthy extraction period (3–6 h), destruction of some compounds that are temperature-sensitive, simultaneous extraction of other components, and large solvent residues [13]. Although advanced extraction techniques are thought to be a useful tool for overcoming the drawbacks of traditional methods, they are not widely used for the commercial manufacture of essential oils due to their high initial investment cost and the requirement of sophisticated technologies. Thus, the parameters that guarantee a good process yield and excellent quality must be in place for the production process of essential oils using conventional extraction procedures [3].

Due to its low initial cost and ongoing operating expenses as well as its environmentally friendly approach, steam distillation extraction is by far the most often used extraction technology [14]. This extraction method still has the disadvantage of having a lesser yield of essential oil. This lower yield is essentially what accounts for the high price of essential oils. Additionally, essential oil produced by steam distillation may degrade principal compounds particularly for those compounds that are temperature-sensitive. As a result, research is still being done to determine how to increase yield without compromising quality of the extracted essential oil [5, 15]. Raw materials quality (plant age, plant part), pretreatments (sun, and shade dried), particle size, mass of feed, and process parameters (extraction time, temperature, steam flow rate, cooling water flow rate, etc.) are the main factors that need to be studied, monitored, and optimized during the steam distillation process in order to get a better yield and good quality essential oils [16].

Prior studies mainly concentrated on the volatile compound profiling [4, 17], biological activity [4, 6, 18], impact of cultivation on chemical composition [10], comparison of various extraction techniques [19], and medicinal and therapeutic usage of cinnamon essential oil [20]. Besides, process parameter optimization for the hydro and steam distillation extraction of Cinnamon essential oil [3, 21] were carried out for limited independent variables. To the best of our knowledge, no studies have been conducted to determine how the extraction temperature, time, and mass of the feed affect the yield and quality of cinnamon leaf essential oil. Additionally, the use of this essential oil as a food preservative against microbial spoilage is an unanswered question that requires further research. Thus, the main aim of this study was to optimize the key steam distillation process parameters that affect the extraction of essential oil from *C. verum* leaf material. Furthermore, elucidating the effect of cinnamon essential oil concentration effects on the microbiological shelf stability of minced beef was another goal of this research.

Materials and methods

Raw material collection, pretreatment and storage

Fresh cinnamon leaf (*Cinnamomum verum Cin.5/82*) was collected from Bebeka Bench Sheko, South West Ethiopia Peoples' Region, Ethiopia (6°53′01.4″N, 35°25′41.9″E). The botanical identification of the sample was previously performed at the Wondo-Genet Agricultural Research Center, under registration number Cin. 5/82. Fresh cinnamon leaves were first washed with tap water to remove any traces of dust and debris. Then, the leaves were shade dried for 21 days at room temperature (24 ± 2 °C) until the moisture content is reduced from 44.42 ± 0.03 to 10.32 ± 0.02%. Finally, it was packed in polyethylene plastic and stored at room temperature until further analysis were carried out.

Steam distillation

Shade dried cinnamon leaves were first placed in a 5 L distillation biomass flask. Externally generated steam was then ejected to the bottom of two serially arranged biomass flasks, the top of which held the ready-to-extract sample (Fig. 1). As the steam passes through the biomass flasks, the essential oil and water vapor rise to the condenser, where they change phases from gas to liquid and are dropped back into the separatory funnel, which is located directly beneath the condenser. After the extraction time was completed, water was drained from the separatory funnel to separate the visible layer of water from the extracted oil. The oil was then dried further by filtering it through anhydrous sodium sulfate. Finally, the obtained essential oils were stored in amber glass bottle at the refrigerator (4 °C) until further analysis.

The extracted essential oil yield was then calculated using Eq. 1

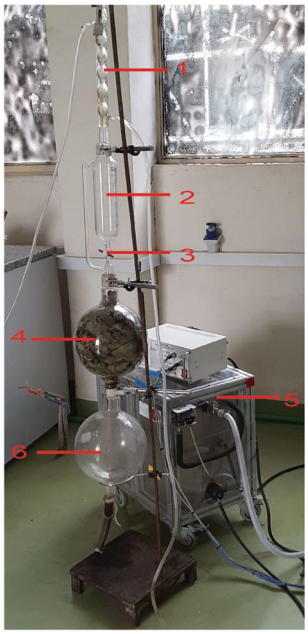

Fig. 1 Extraction of cinnamon essential oil via steam distillation with an experimental setup comprising (1) Condenser, (2) Separatory funnel, (3) Separatory funnel valve, (4) Steam generator, (5) Sample holder biomass flask, and (6) Steam injection biomass flask

$$\text{Yeild (\%)} = \frac{\text{Mass of extracted oil (g)}}{\text{Intial plant biomass (g)}} * 100 \quad (1)$$

Process optimization

Response surface methodology (RSM) was used to study the effect of extraction time (A), temperature (B), and feed mass (C) on extraction yield (Y_1) and cinnamaldehyde concentration (Y_2). RSM design with the uncoded and coded levels are tabulated in Table 1. Central composite design (CCD) was used to design the experiment which gives a total of twenty randomized treatments, including eight fractional factorial points, six axial points, and six central points.

Volatile compound analysis

The volatile aromatic component profile of extracted cinnamon essential oil was carried out utilizing gas chromatography tandem with mass spectrometry (Agilent, 7890B/G7038A GC/MS System) methods adapted from [22]. Briefly, 1 µL of essential oil sample was ejected to GC capillary column (HP-5MS UI, 30 m, 0.250 mm, 0.25 µm) with the help of auto sampler and separation was facilitated using helium as carrier gas at flow rate of 1.0 mL/min. The oven temperature of GC was programmed as follows: 120 ℃ for 3 min isothermal, 120–260 ℃ with a rate of 10 °C /min, 8 min isothermal. The quadrupole mass spectrometer was operated at a split ratio of (1:20), and the acquisition scan ranged was set from 50 to 550 m/z within electron impact mass spectra of 70 eV ionization energy. The final identification of volatile compounds was performed by comparing retention indices and mass spectra with the GCMS library (NIST) and literature.

Total phenolic content

The total phenolic content for each extract was determined spectrophotometrically using Folin–Ciocalteu (FC) procedure as described in [23]. About 100µL of extracted Cinnamon essential oil was mixed with 100µLof methanol, and 0.1 mL of mixture was transferred into a volumetric flask and diluted with 0.5mL of deionized water. Test tubes were filled with 0.2 mL of the extract and 0.5 mL of Folin-reagent. Ciocalteu's (diluted

Table 1 The five levels of the independent variables used in central composite design

	Symbol	Coded level				
		− α	− 1	0	+1	+ α
Time (min)	A	89.319	120	165	210	240.88
Temperature (°C)	B	103.296	105	110	115	111.704
Feed mass (g)	C	401.928	300	450	600	100.928

with water 1:10). After being held in the dark for 5 min, the solution received 1 mL of sodium carbonate (7.5% w/v). The tubes were once more maintained in the dark for an hour with parafilm on top. Using a spectrophotometer UV-vis (Jasco V-530), absorption at 765 nm was measured and compared to a calibration curve for gallic acid. The gallic acid standard reference curve was constructed for the following concentrations, in turn: 0, 20, 40, 60, and 80 μg mL^{-1}. The outcomes were given in mg gallic acid/g of dried material.

DPPH radical scavenging

Using the stable radical DPPH, the antioxidant activity was determined in terms of its capacity to donate hydrogen or scavenge free radicals. The Blois, (1958) method was employed to conduct the experiments [24]. The rationale behind this colorimetric assay is that as the radical concentration in a solution decreases, so does the absorbance at 517 nm. Two mL aqueous methanolic stock solution of cinnamon essential oil extract and 2 mL of a 1 mM DPPH solution was added to a test tube. The tube was then maintained in the dark for an hour with parafilm on top. Finally, spectrophotometer UV-vis (Jasco V-530) was used to measure the absorbance at 517 nm wave length and compare the results to a calibration curve for ascorbic acid. The assay was performed in triplicate. The inhibition percentage of the DPPH radical was calculated using the Eq. 2.

$$I\ (\%) = \frac{A_0\text{-}A}{A_0} * 100 \quad (2)$$

where I = DPPH inhibition (%), A_0 = absorbance of control sample (t = 0 h) and A = absorbance of a tested sample at the end of the reaction (t = 1 h).

Antibacterial activity test

The Agar well diffusion assay was used to assess the antibacterial activity of the cinnamon leaf essential oils [25]. A total of five most common foodborne bacterial pathogens (*Staphylococcus aureus (*ATCC 25,923), *Listeria monocytogenes (*ATCC 19115), *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27,853), *Acinetobactor baumannii* (ATCC 19606)) were first obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. Briefly, each bacterium was first subcultured in nutrient agar at 37 ℃ for 24 h. Hundred μL of standardized inoculums of the test microorganisms were spread over sterile Muller-Hinton Agar for bacteria. Agar was then cut into 8 mm diameter wells with a sterile cork-borer, and 100 μL of the essential oils with different concentrations were then placed into different well. The plates were first incubated at room temperature for one hour to ensure appropriate oil diffusion into the agar, and then at 37 ℃ for 24 h for three days. Each assay was performed in triplicates. As Ashraf et al. had done [26], the inhibition zone was eventually determined in millimeters.

Storage stability test

The methods used by Burt [27] were utilized to analyze the microbiological shelf stability of minced beef samples. Ten samples of each treatment were prepared by mixing 50 g of minced beef with various cinnamon essential oil concentrations. The treated samples were then packed in sterile polyethylene bags and stored at refrigerated (4 °C) condition. During the time series analysis, a ten-gram sample from each treatment was hygienically taken and added to stomacher bags containing 90 mL of 0.1% saline water for thorough homogenization for about one minute at 25 °C. Serial dilutions of each sample were then created in 0.1% saline water, and a duplicate 1 ml sample of each dilution was spread into Plate Count Agar (PCA). The total viable bacteria were then enumerated after incubation at 37 ℃ for 48 h. Microbial colonies were enumerated as total viable count for the plats that had colonies of between 30 and 300.

Statistical analysis

Design Expert Software (V. 13) was used to statistically assess the experimental data. The best-fitting polynomial model was chosen by comparing a number of statistical metrics (lack-of-fit, coefficient of variation, and predicted and adjusted correlation coefficients). Furthermore, the analysis of variance of significant differences were identified by computing the F-value for the probabilities of 0.01, 0.1, and 0.5.

Result and discussion

Optimization of cinnamon leaf essential oil extraction

Steam distillation method is widely used for extracting essential oils from various plant sources due to their economic feasibility issues. However, it has limitations in terms of product quality degradation, particular for temperature sensitive active ingredients. Besides, extraction of essential oil from the plant source using steam or hydro-distillation methods is time and energy consuming process. Basically, if steam distillation is used as an extraction technique, finding quality steam at a lower temperature is very challenging unless another pressure-reducing system is employed. As a result, further lowering of the temperature during conventional steam distillation is nearly impossible. However, by reducing exposure time, and contact surface between the steam and the plant material, acceptable essential oil quality can be maintained. These issues could be partially addressed

by manipulating the extraction process parameters. Thus, in this study, RSM optimization technique was applied for tweaking independent extraction parameters with an objective to achieve a higher yield without major compromisation on the quality of the extracted essential oil. However, essential oils extraction using stream distillation is one of the complex processes which applies both heat and mass transfer unit-operations. Thus, to optimize and predict such process using white-box model is very complicated task. Rather, with all due its limitations, selecting and optimizing the influential process parameters for maximizing the yield and quality of essential oil using RSM is the cost-effective alternative means [28]. It is a mathematical and statistical technique for studying and optimizing multivariable systems by determining the relationship between the set of independent variables and the response. Extraction time, temperature, and feed mass were the independent variables optimized in this study for maximizing yield and quality. These parameters had shown a significant effect on the aforementioned response for both steam and hydro-distillation extraction process. Particularly, increasing extraction time and temperature had shown an increase in the yield of essential oil [29]. Basically, cinnamaldehyde and eugenol were major compounds found in all cinnamon leaf extract. Nevertheless, in this study, the relative concentration of eugenol was almost similar for all treatment samples. Thus, cinnamaldehyde was used to compare the quality of the extracted cinnamon leaf essential oil. The effect of independent variables on the response (experimental and predicted) values are tabulated in Table 2.

Influence of independent parameters on the essential oil yield

Taking other variables into account, each independent variable had a significant ($P < 0.001$) effect on the extraction yield of cinnamon leaf essential oil. Extraction time, one of the independent variables, had a significant effect on cinnamon leaf essential oil yield at both the linear and quadratic levels (Table 3). This could be explained by an increase in mass transfer, which will allow the system to approach equilibrium as the extraction time increases. Similarly, both the linear and quadratic levels of extraction temperature showed a significant effect on essential oil yield. This could be because the extraction temperature facilitates rapturing the plant structure to release the essential oil, which improves the rate of diffusion during the extraction process [30]. Feed mass had also a significant effect on the extraction yield both at linear and quadratic level (Table 3).

Predicted response for the cinnamon essential oil yield was expressed by second-order polynomial regression equation in terms of coded values (Eq. 3):

Table 2 Experimental and predicted values of extraction yield and Cinnamaldehyde concentration

Run	Time (min)	Temperature (℃)	Feed Mass (g)	Yield (%)		Cinnamaldehyde (%)	
				Experimental	Predicted	Experimental	Predicted
1	120	115	300	2.41	2.46	26.00	26.03
2	165	110	197.731	2.24	2.19	25.87	25.52
3	165	110	450	2.98	3.07	25.93	25.77
4	210	115	300	2.49	2.55	26.5	26.71
5	240.681	110	450	3.14	3.03	25.97	25.69
6	165	101.591	450	2.49	2.46	35.41	34.44
7	89.3193	110	450	2.61	2.59	28.31	27.52
8	165	110	702.269	2.98	2.90	33.31	32.59
9	120	105	300	2.14	2.13	26.85	27.64
10	120	115	600	2.80	2.84	26.40	26.98
11	165	110	450	3.10	3.07	25.86	25.77
12	120	105	600	2.51	2.54	36.33	36.88
13	165	110	450	3.10	3.07	25.23	25.77
14	210	115	600	2.89	2.99	25.89	25.86
15	165	118.409	450	2.84	2.74	26.32	26.22
16	165	110	450	2.99	3.07	26.21	25.77
17	210	105	600	2.94	2.98	33.31	34.04
18	165	110	450	3.11	3.07	25.21	25.77
19	210	105	300	2.45	2.51	26.41	26.59
20	165	110	450	3.11	3.07	25.99	25.77

Table 3 The regression coefficients values of the responses with their p-values

Variables	Yield (g/g)		Cinnamaldehyde (%)	
	Regression coefficient	*p-values*	Regression Coefficient	*p-values*
Intercept	3.07	0.0001	25.77	0.0001
A-Time	0.1319	0.0002	− 0.5422	0.0180
B-Temperature	0.0834	0.0053	− 2.45	0.0001
C-Feed Mass	0.2119	0.0001	2.10	0.0001
AB	− 0.0712	0.0429	0.4312	0.1163
AC	0.0163	0.6085	− 0.4488	0.1039
BC	− 0.0088	0.7816	− 2.07	0.0001
A^2	− 0.0918	0.0025	0.2957	0.1446
B^2	− 0.1661	0.0001	1.61	0.0001
C^2	− 0.1855	0.0001	1.16	0.0001
R^2	0.9609		0.9791	

$$Yield = -89.311 + 0.0516A + 1.5355B + 0.0097C - 0.0003AB + 0.0002AC - 0.0001BC - 0.0001A^2 - 0.0066B^2 - 0.0001C^2 \quad (3)$$

Where: A = Extraction time temperature, B = Extraction temperature, and C = Feed mass.

Statistical analysis of variance (ANOVA) revealed that, the experimental data could be best represented by quadratic polynomial model with coefficient of determination (R^2) values of 0.9809 for cinnamon essential oil yield (Table 3). This R^2 values, which was closed to unity, indicate that our quadratic polynomial model adequately describes the system. The significance level for quadratic polynomial model coefficients tabulated in Table 3 was calculated using analysis of variance (ANOVA).

To better visualize the interaction effect of independent variables on the yield of cinnamon leaf essential oil, 3D plots were employed, with one independent variable held constant while the other two variables were varied (Fig. 2). When the input mass was modest (300 g), increasing the extraction time resulted in an increase in extraction yield. This positive effect could be due to the creation of an environment that results in increased contact time between the solvent and the solute. The same is true for biomass increase, even at shorter extraction time (120 min). The combination of time and biomass feed generates a high essential oil yield (Fig. 2). Nonetheless, a slight decrease in essential oil yield was observed for the combination of longer extraction time and higher feed mass. This could be due to the increased mass transfer resistance caused by the higher feed mass. Similarly, at low temperatures (105 °C), increasing the extraction time resulted in a higher extraction yield. Higher extraction temperature at a lower extraction time resulted in an increase in essential oil production (120 min). This could be attributed to higher extraction temperatures causing greater disruption of plant cells. The combined increment of time and temperature had shown high essential oil yield. However, longer extraction time and higher temperature showed a trivial decreases in the cinnamon essential oil yield (Fig. 2B). A loss of some essential oil in the condenser due to solute-containing steam flow could explain the relatively lower essential oil at longer time and higher temperature extraction conditions. The increase in feed mass and temperature had also resulted in an increase in the yield of cinnamon essential oil (Fig. 2C). However, increasing feed mass and temperature resulted in a drop in essential oil yield. This could be owing to higher feed mass mass transfer resistance or higher steam temperature, which could cause a loss of volatiles. During the study of optimizing oil and pectin extraction from orange peels, Fakayode and Abobi, [31] also discovered a similar result.

The perturbation plots were used to compare the effects of extraction time, temperature and feed mass at a particular point in the design space (Fig. 3A). The plot is specifically used to demonstrate the yield of cinnamon essential oil yield by varying only one independent variable over its range while holding all the other variables constant. By default, Design-Expert sets the reference at the midpoint (i.e. 165 min, 110 ℃ and 450 g) of all the independent variables. As the extraction time, temperature and feed mass were increased the extraction yield also increased, and vice versa. The feed mass, in particular, showed a greater increase and reduction in essential oil yield as it went away from the center (Fig. 3A). This could be directly related to the solute concentration in the extraction system.

Influence of independent parameters on the quality of essential oil

The two primary compounds found in cinnamon leaf essential oil are eugenol and cinnamaldehyde [8]. These two crucial components are responsible for the essential oil's distinct aroma and flavor, as well as its unique therapeutic characteristics. Cinnamaldehyde was used in this study to optimize the quality of cinnamon essential oil since the concentration of eugenol was much higher and constant across all samples. The extraction temperature and feed mass had a significant effect on the relative concentration of cinnamaldehyde at both the linear and quadratic levels ($P < 0.001$). Although extraction time had a significant effect on essential oil quality at the linear

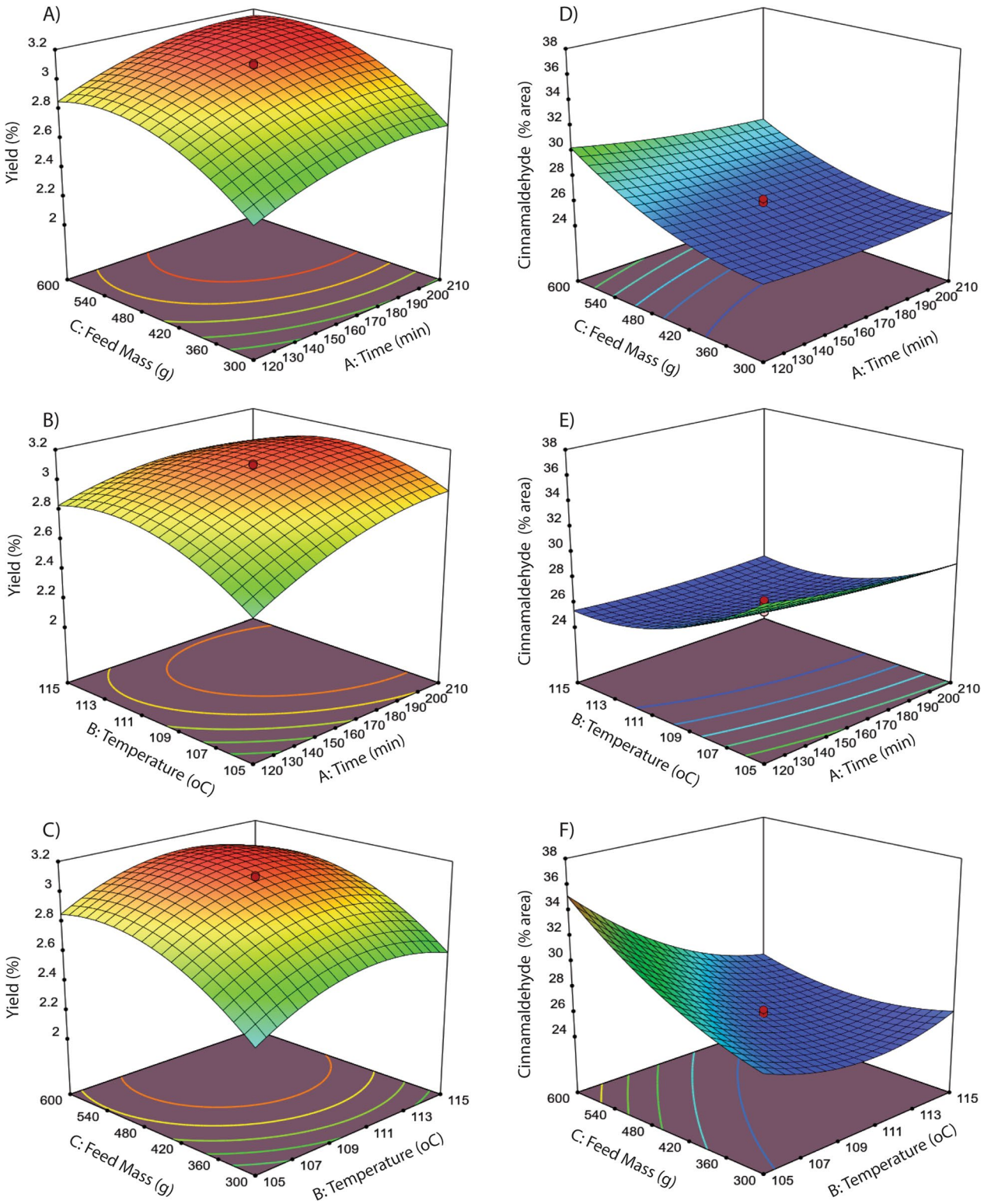

Fig. 2 Response surface plots demonstrating the interaction between **A** Feed mass (g) and Time (min), **B** Temperature (℃) and Time (min), **C** Feed mass (g) and Temperature (℃) on Yield (%) of cinnamon essential oil; **D** Feed mass (g) and Time (min), **E** Temperature (℃) and Time (min), **F** Feed mass (g) and Temperature (℃) on cinnamaldehyde (% area)

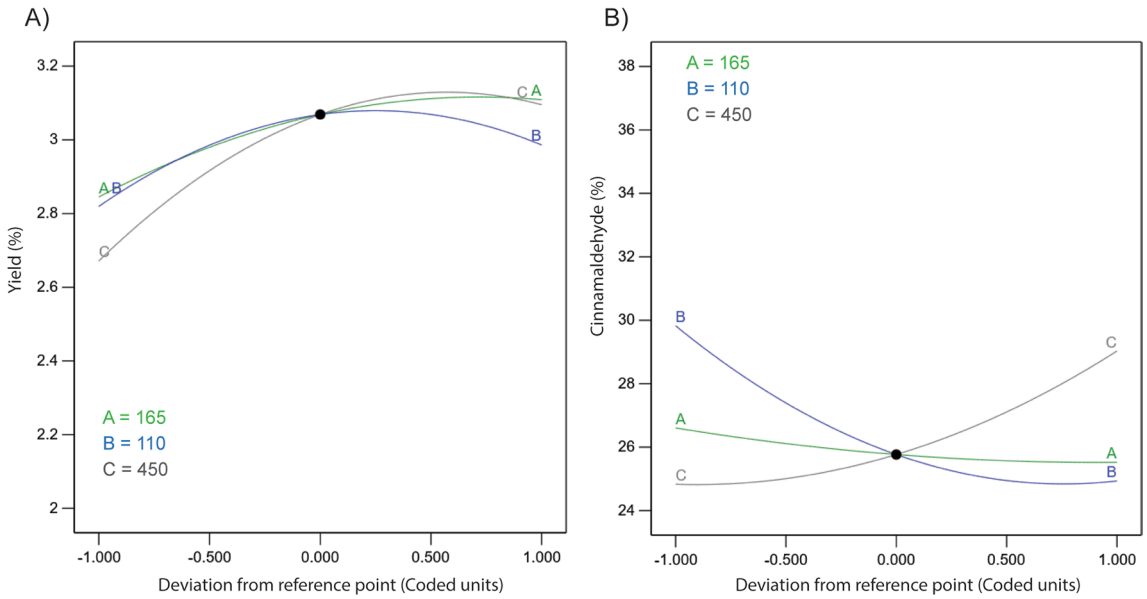

Fig. 3 Perturbation plot illustrating the change effect of the independent variables from maximum to minimum values on **A** Yield (%), and **B** cinnamaldehyde (% area)

level ($P < 0.05$), it lacked a significant ($P > 0.05$) effect at the quadratic level (Table 3).

The relative cinnamaldehyde concentration of cinnamon essential oil yield can be predicted by the following second-order polynomial regression equation in terms of coded values (Eq. 4):

$$\begin{aligned} &\text{Cinnamaldehyde} \\ &= 763.229 - 0.2412A - 13.753B + 0.2826C \\ &\quad + 0.0019AB - 0.0066AC - 0.0028BC \\ &\quad + 0.0015A^2 + 0.0645B^2 + 0.0052C^2 \end{aligned} \tag{4}$$

Where: A = Extraction time temperature, B = Extraction temperature, and C = Feed mass.

A 3D surface plot was used to demonstrate the interaction effect of independent variables on the relative concentration of cinnamaldehyde (Fig. 2). The independent variable of extraction time had a weak interaction effect with either extraction temperature or feed mass (Fig. 2D, E). As a result, the influence of time on the quality of cinnamon essential oil (cinnamaldehyde) was not significant, at least within the time period designated for this investigation. This could be owing to the volatile nature of the cinnamaldehyde component, which causes it to volatilize at an early stage of the process [16]. While the extraction period is held constant, increasing the feed mass increases the relative concentration of cinnamaldehyde (Fig. 2D). Increasing the feed mass resulted in a higher relative cinnamaldehyde concentration regardless of extraction time or temperature (Fig. 2D, F). This could be due to the increased availability of solute concentration caused by the increase in feed mass. Furthermore, when the extraction period was extended, the bigger biomass showed less quality degradation than the smaller feed quantity. A significant ($P < 0.05$) interaction effect was also seen when extraction temperature was paired with either extraction time or feed mass (Table 3). The relative concentration of cinnamaldehyde was particularly reduced at higher temperature processing with longer extraction time (Fig. 2E). Thermal-induced decomposition caused by greater extraction temperatures could account for the lower relative concentration of cinnamaldehyde. Besides, the detrimental effect of extraction temperature on the quality of cinnamon essential oil was exacerbated by the reduced feed mass compared to its counterpart (Fig. 2F). This could be due to a reduction in direct contact between the high-temperature steam and the solute found in bigger biomass.

To better visualize the effect of each independent variable on the cinnamon essential oil quality as it moved away from the mid-point while all other parameters stayed constant at specific reference value, a perturbation plot was used (Fig. 3B). The relative concentration of cinnamaldehyde increases as the temperature decreases from the center point (reference specified by Design-expert software), and the opposite is fully true. Meanwhile, when the feed mass grew from the reference point, so did the relative concentration of cinnamaldehyde, and vice versa.

Optimization of independent variables

Different optimum solutions for yield and quality of cinnamon leaf essential oil were predicted by using design expert software V.13. Maximization goals were selected for the optimization of both cinnamon leaf essential oil yield and cinnamaldehyde relative concentration. For the optimal values for various independent variable combinations, fifty-four distinct numerical solutions were produced. From the 54 solutions listed, a process parameter combination of 175.43 min, 105 ℃, and 600 g time, temperature, and feed mass were chosen as the optimum process parameter combination for the greatest desirability of 0.71, respectively. At these optimized extraction conditions, the response values were 2.9% extraction yield and 34.6% relative cinnamaldehyde concentration.

Validation of the developed RSM models

The adequacy of the model for predicting response values was tested using optimized cinnamon leaf essential oil extraction. This was accomplished by carrying out the extraction experiment at optimal conditions. At optimum processing conditions, the predicted extraction yield was 2.9% and the cinnamaldehyde relative concentration was 34.6%. Meanwhile, during the validation experiment at optimized extraction conditions, the extraction yield was 0.289 and the cinnamaldehyde relative concentration was 33.94%. This finding shows that the experimental response values were very near to that of the projected values (Table 4).

Chemical composition and antioxidant activity of cinnamon essential oil

For additional functionality study, cinnamon essential oil that was extracted utilizing the steam distillation process under optimum conditions was employed. The essential oil content was evaluated using GC–MS before further study. This GC–MS study found twenty-four compounds in total. The first 10 of these compounds account for more than 98% of the total relative area coverage. When compared to the other compounds, eugenol was by far the most prevalent (60.68%). It has been established that eugenol is first produced in the cinnamon leaves and that the barks make up a very little portion on the biosynthesis process. This result is actually in a good agreement with the previous results in which, eugenol is the primary component of essential oils from cinnamon leaf oils. Cinnamaldehyde (33.94%) was the second significant component found in cinnamon leaf essential oil extract (Table 5). Similar dominance of these two compounds were also observed for essential oil extracted from five cinnamon leaves [10]. Other than this two the other compounds shown a lower relative area coverage (< 2%) for the GC–MS analysis. The percentage area coverage for the other compounds, excluding these two, was lower (2%) for the GC–MS analysis. It is nevertheless important to be aware that the composition of essential oils might change depending on the timing of harvest, the country of origin, the stage of a plant's development, and market storage conditions [32].

Total phenol, total flavonoid, and DPPH free radical scavenging potential of cinnamon essential oil extracted at optimum condition were analyzed to assess the antioxidant activity. This antioxidant activity's mode of action is to reduce oxidative stress in the body by scavenging the generated free radicals of ROS and RNS (reactive nitrogen species). Particularly phenolic compounds have a reputation for acting as antioxidants due to both their propensity to donate electrons or hydrogen as well as the fact that they are stable radical intermediates. In this study, cinnamon essential oil showed a good antioxidant activity for the aforementioned antioxidant analysis (Table 5). This good antioxidant activity by major comes from the principal compounds of cinnamon essential oil. Particularly, cinnamaldehyde demonstrated a good free radical–scavenging activities in the previous experiments [30]. Similar, good antioxidant activity was seen for in vitro cinnamon essential oil antioxidant activity study [17, 33].

Antibacterial activity and minimum inhibitory concentration

Essential oils represent a potential source of new antibacterial compounds, especially against some genera of bacteria that cause food deterioration. The antibacterial

Table 4 Optimum conditions, predicted and experimental values of the response variables

Optimum conditions	Coded levels	Values
Extraction time (min)	0.43	175.43
Extraction temperature (℃)	− 1	105
Feed Mass (g)	− 1	600
Response variables	**Predicted values**	**Experimental values**
Yield (%)	2.90	2.915 ± 0.72
Cinnamaldehyde concentration (% area)	34.60	33.94 ± 0.61

Table 5 Chemical composition and antioxidant activity optimally extracted cinnamon leaf essential oil

S. N	Name of the compound	Chemical composition				
		Molecular Formula	Rt (min.)	Retention index (RI)		% Area
				RI	Literature RI	
1	α-Pinene	$C_{10}H_{16}$	5.553	896	917	0.07
2	α-Phellandrene	$C_{10}H_{16}$	7.266	986	996	0.02
3	O-Cymene	$C_{10}H_{14}$	7.796	921		0.45
4	Limonene	$C_{10}H_{16}$	7.918	945	995	1.03
5	Linalool	C_9H_8O	9.862	1039	1090	1.29
6	Cinnamaldehyde	C_9H_8O	14.629	1260	1266	33.94
7	Eugenol	$C_{10}H_{12}O_2$	16.967	966	1373	60.68
8	α-Copaene	$C_{15}H_{24}$	17.439	913	1372	0.16
9	Caryophyllene	$C_{15}H_{24}$	18.573	892	1415	0.38
10	Benzyl benzoate	$C_{14}H_{12}O_2$	26.82	1479		0.21
					Antioxidant activity	
	Total phenols				70.8 ± 0.52 mg GAE/g	
	Total flavonoids				36.50 ± 0.36 mg QE/g	
	DPPH				44.65 ± 0.69 IC_{50} µg/mL	

Table spaces left blank are for RI values that have not been discovered in the literature under similar operating conditions

activity of cinnamon essential oil was investigated in this work using invitro disc diffusion assay. Figure 3 illustrates the antibacterial activity of various concentrations of cinnamon essential oil against five selected foodborne bacterium species [*Staphylococcus aureus (*ATCC 25923), *Listeria monocytogenes* (ATCC 19115), *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa (*ATCC 27853), *Acinetobactor baumannii* (ATCC 19606)]. These bacteria were chosen based on their high prevalence in most Ethiopian foods [34] as well as the variations in their membrane structures, which play a significant role in how they react to the applied essential oil.

Given the variety of chemical component present in cinnamon essential oils, the method of action to prevent microbial growth could not be attributed to a single mechanism and may instead target several activities in the cell. Cinnamaldehyde's carbonyl group is predicted to bind to proteins and prevent the bacterial amino acid decarboxylases from doing their job. Moreover, because essential oils and their components are hydrophobic, they can permeate the lipids of the mitochondria and bacterial cell membrane, upsetting the structure of the cell and making it more permeable. Cell death will occur as a result of this significant bacterial cell leakage or the loss of vital substances and ions.

The minimal inhibition concentration for the applied essential oil concentrations, however, varied from bacterium to bacteria as did the level of the inhibition zone. Regardless of the bacterial family, the inhibitory effect grows as the essential oil concentration is increased (Fig. 4, Additional file 1: Fig. S2). In this study, the lowest minimal inhibition concentration was observed for *S. aureus* at 0.2% (v/v). while, the highest minimum inhibitory concentration was observed for the *E. coli* (Fig. 4). Besides, all the gram-negative bacteria in this study showed the higher essential oil concentration as compared to the gram-positive bacteria. This difference is most likely due to the intricacy of gram-negative bacteria's double membrane-containing cell envelope, as contrast to gram-positive bacteria's single membrane glycoprotein or membrane-glycoprotein-based structures. Furthermore, the highest inhibition zone (14 mm) was observed for the disc inoculated with *S. aureus* at 0.8% (v/v) cinnamon essential oil concentration (Fig. 4). These inhibition zones are smaller than those reported in earlier studies [35] that tested the antibacterial activity of crude cinnamon essential oil (20 mm), however the concentration of the applied oil was very much higher as compared to this study as well as recommended dosage applied to the food.

Effect of essential oil concentrations on storage stability of minced beef

When total viable microbiological counts (TVC) in any food product reach 7 log CFU/g, the product is considered spoiled [36]. Figure 4 depicts the TVC dynamics of minced beef seasoned with cinnamon essential oil and

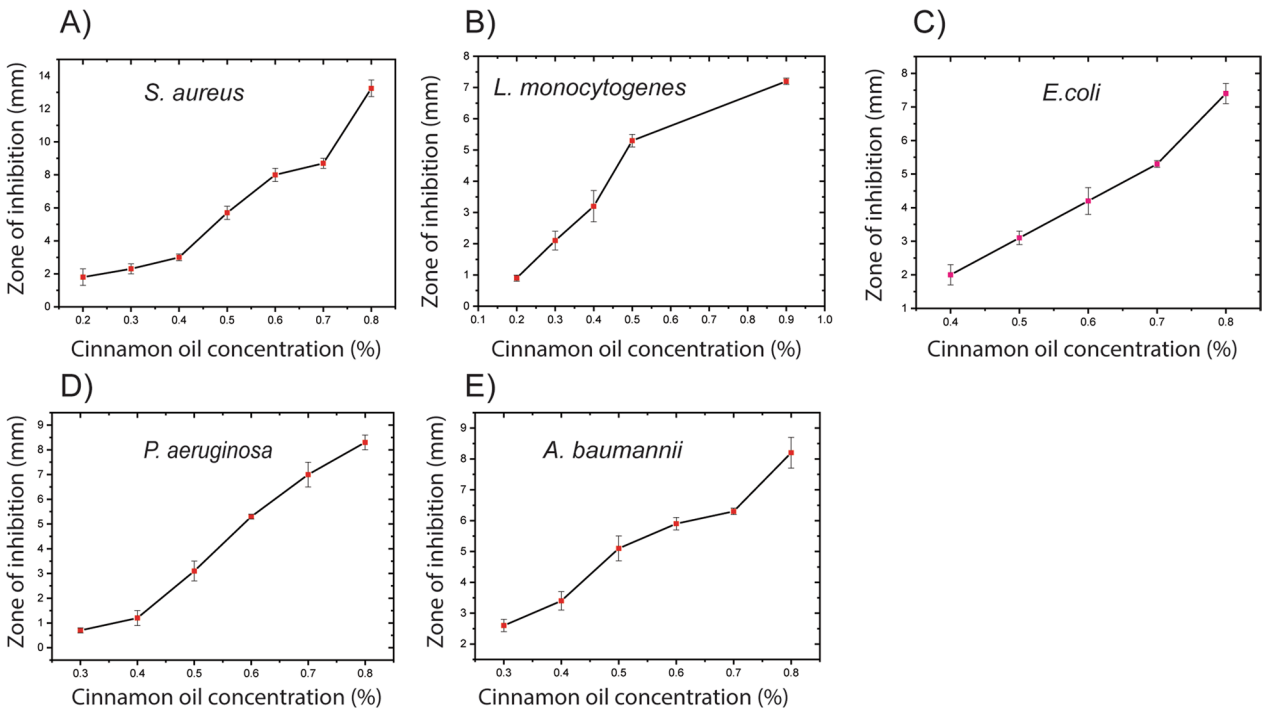

Fig. 4 Zone of microbial inhibition for different concentrations of cinnamon essential oil against **A** *S.auerus*, **B** *L. monocytogenes*, **C** *E.coli*, **D** *P. aeruginosa*, **E** *A.baumannii* bacterial species

control samples stored at 4 ℃ for 21 days. The raw beef utilized in this investigation had an initial TVC of 3.82 log CFU/g. The use of cinnamon leaf essential oil significantly decreased the growth rate of TVC in minced beef samples ($P < 0.05$). More particular, when a higher concentration of cinnamon essential oils was added to minced beef, the rate of microbial growth decline was faster (Fig. 5).

However, increasing the essential oil concentration to more than 1% may have a detrimental influence on the sensory quality of the food [37]. Nonetheless, even at 0.5% concentration of cinnamon essential oil treated samples were within the acceptable range for the twenty-one-day refrigerated storage in this investigation. Previous research on the evaluation of effectiveness of cinnamon essential oil on food deterioration microorganisms yielded similar results [38, 39]. The control sample, on the other

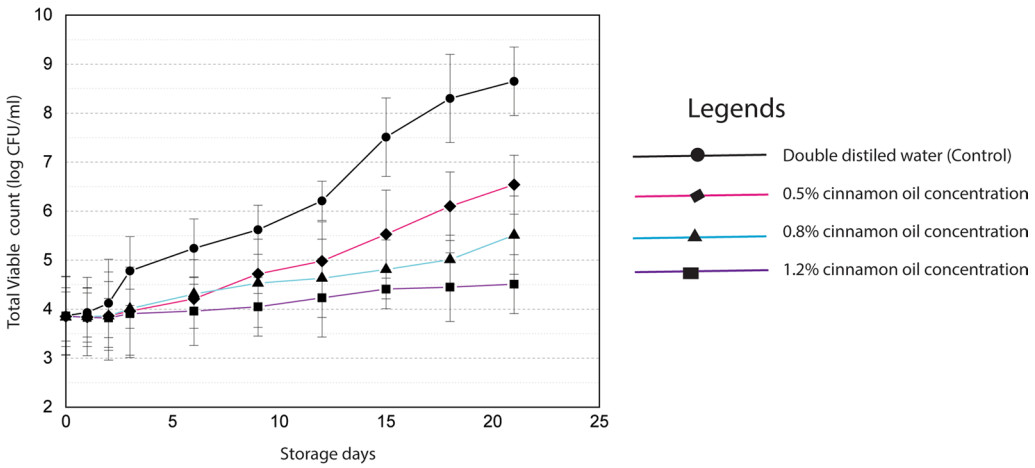

Fig. 5 Effect of cinnamon essential oil concentrations on the microbial shelf stability of minced beef

hand, showed a considerable growth rate after the fourth day of storage and exceeded the upper limit of 7 log CFU/g after fifteen days of refrigerated storage (Fig. 5).

However, increasing the essential oil concentration of above 1% might have a negative impact on the sensorial attribute of the food products [37]. Nevertheless, in this study even at 0.5% concentrate of cinnamon essential oil treated samples was with the acceptable range for the twenty-one days refrigerated storage. Previous studies on the evaluation of cinnamon essential effectiveness on the food spoilage microorganisms had also reported similar findings. Whereas, the control sample had shown a significant growth rate after fourth day storage and cross the maximum limit of 7 log CFU/g after fifteen days of refrigerated storage (Fig. 5). Several factors, including cell wall rupture brought on by bioactive substances, cytoplasmic membrane disruption, cellular component stress brought on by leakage, altered fatty acid and phospholipid constituents, affecting RNA and DNA formation, and wrecking protein translocation, are cited as the explanations for this inhibition of microbial growth.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-023-00798-y.

Additional file 1: Fig. S1. Fresh and shade-dried cinnamon leaves. **Fig. S2.** Inhibition zones of the Cinnamon essential oil against the common foodborne microorganisms **A** *Acinetobacter baumannii* (ATCC 19606), **B** *Escherichia coli* (ATCC 25922), **C** *Staphylococcus aureus* (ATCC 25923), **D** *Listeria monocytogenes* (ATCC 19115), **E** *Pseudomonas aeruginosa* (ATCC 27853).

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Author contributions
RMY: conceptualization, Methodology, investigation, writing—original draft. HA: conceptualization, writing—review and editing, supervision. FMI: conceptualization, supervision. EGF: data curation, formal analysis, visualization, software, writing—original draft.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competinginterests

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