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Evaluation of *Ocimum sanctum* Essential Oil as Potential Preservative for Fermented Dairy Products

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Ocimum sanctum **essential oil was evaluated for its antimicrobial activity to use it as natural preservative for fermented dairy products against food borne pathogens and its compatibility with starter cultures. Antimicrobial potential was evaluated by disc and agar well assay alongwith determination of its minimal inhibitory concentration (MIC). Disc and agar well assay revealed maximum inhibition of pathogens as compared to starter cultures. Concentration range of 0.1-2.0 μL mL^{-1} was used to test MIC of all target microorganisms. Concentrations of 0.4-0.6 μL mL^{-1} was effective in inhibiting the growth of above pathogens microorganisms whereas 3-4 times higher concentration i.e. 1.8 μL mL^{-1} was required for bacteriostatic action against dairy starter cultures indicating its compatibility in broth. The compatibility of dairy cultures with 0.1-2.0 μL mL^{-1} concentration of essential oil were also determined in milk matrix (as dahi and yoghurt fermented product model) by measuring change in pH, titratable acidity and curd setting time. Concentration of 0.5-1.0 μL mL^{-1} of *Ocimum sanctum* essential oil was found to be safe which can be directly used for preservation purpose without affecting starter cultures activity vis-α-vis quality of fermented products.**

Keywords: *Ocimum sanctum* Linn. (Tulsi); essential oil; minimal inhibitory concentration; pathogens; dairy starters.

India is known for its ancient civilization and for its traditional medicinal knowledge, i.e. "*Ayurveda*", unparalleled in the world for its negligible or no side effects as compared to allopathic drugs. Historically, people are aware of beneficial potentials of various herbs in human health and inhibitory activity towards pathogens as well as spoilage microflora. Amongst, the herbal treasure of India *Tulsi* or holy basil (*Ocimum sanctum* Linn.), *Mentha piperita*, *Cinnamon*, *Licorice* etc., possess antimicrobial potential against food spoilage and pathogenic microbes. *O. sanctum* inhibits bacteria (*Arthrobacter globiformis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas* spp. *Staphylococcus aureus*, *Staphylococcus albus*, *Salmonella typhi* and *Vibrio cholera*) (Rao and Nigam, 1970; Dey and Choudhary, 1984; Joshi, 2013). Antimicrobial activity of *Ocimum sanctum* have been reported to be higher as compared to commonly available other species of *Ocimum* (*O. canum*, *O. gratissimum*, and *O. basilicum*) in India (Sinha and Gulati, 1990). The aqueous and alcoholic extract and seed oil of *Tulsi* have shown potential antimicrobial properties against enteric pathogens (Geeta *et al*., 2001; Singh *et al*., 2005). Consumption of contaminated dairy foods with pathogens such as *Listeria monocytogenes*, *B. cereus*, *S. aureus*, toxigenic *E. coli* and *S. typhi* leads to several outbreaks, which have a wide impact on economic and public health worldwide (Gandhi and Chikindas, 2007). These pathogens are widely

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adapted to range of environmental conditions as well as in a large variety of raw and processed milk and dairy products such as cheese, yoghurt and other fermented foods. Currently available technologies for preservation and shelf life extension of food include enormous use of chemical preservatives and heat processing techniques. Awareness towards green consumerism and ineffective elimination of pathogens and spoilage microorganisms from these products or microbial spoilages leads to development of alternate preservation techniques such as incorporation of naturally derived antimicrobial ingredients like essential oils (EOs). They also have Generally Regarded As Safe (GRAS) status and their broad spectrum inhibiting action against both Gram-positive and Gram negative microorganisms are gaining attention for its applicability in food and dairy products (Oussalah *et al.*, 2006; Burt, 2004). Antimicrobial action of essential oil involves partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable for leakage of ions, ATP and secondary cell messengers etc. Essential oil such as *O. sanctum* oil has LD_{50} value of 4571.43 µL kg^{-1} on mice which suggests its non-mammalian toxic nature (Kumar *et al*., 2010). Also its major components like eugenol are safe for use (Burt, 2004). The antibacterial activity of EO can be more effective towards foodborne pathogens by using essential oils in combination with lactic acid bacteria (LAB) and their metabolites. The essential oils exhibits high MIC values for LAB as compared to pathogens and spoilage microorganisms (Shipradeep *et al.*, 2012; Moritz *et al.*, 2012). Due to this difference between LAB and pathogen vulnerability, essential oils may find application as antimicrobial agent as natural preservatives in food and dairy products without causing much instability of products thereby improving its functionality (Tassou *et al.*, 1995; Singh et al., 2011). Thus, the present study was aimed to determine the inhibitory activity of *O. sanctum* EOs against foodborne pathogens and spoilage microorganisms like *S. aureus*, *L. monocytogenes*, *B. cereus*, *E. coli* and *S. typhi* relative to starter microorganisms and its effect on performance of yogurt and dahi starter culture for preparation of dairy products.

MATERIALS AND METHODS

Milk and essential oils

Fresh buffalo milk was collected from cattle yard of ICAR-National Dairy Research Institute, Karnal, (Haryana), India. *O. sanctum* essential oil (batch 11006; specific gravity at 20°C, 0.891-0.954; refractive index at 20°C, 1.47-1.56; extracted through steam distillation) for the present investigation was procured from Moksha lifestyle products, New Delhi. Milk was stored at refrigeration temperature while EO was placed at room temperature in dark place until use.

Indicator microorganisms and media

Freeze dried ampoules of *Staphylococcus aureus* and *Salmonella typhi* culture and all starter microorganisms listed in Table 1 were procured from National Collection of Dairy Cultures, (NCDC) Karnal, Haryana. American Type Culture Collection (ATCC) strains were obtained from Food safety and Biosensor Laboratory, ICAR-National Dairy Research Institute, Karnal- 132001, Haryana (India). All the bacterial cultures were activated and maintained in glycerol stocks at -80±1°C containing 20% glycerol. All chemicals and culture media were purchased from Hi-Media Laboratories, Mumbai, India.

Antimicrobial activity of essential oil

Antimicrobial activity of essential oils against selected pathogen and spoilage microorganism as well as compatibility with lactic cultures was determined by two methods *viz*., disc assay and agar well diffusion method (Table. 2).

Disc diffusion method

Inhibitory potential of the essential oil against microorganisms was evaluated according to disc diffusion method reported by Sfeir *et al*. (2013).Freshly prepared suspension of each microorganism (10^5-10^6 CFU/mL) was spread on solidified agar (tryptone glucose yeast extract agar for pathogenic or spoilage microorganisms and MRS or M-17 for starter microorganisms). Sterile paper disc (4 mm diameter) was wetted with 5 µL of essential oil. The disc was placed onto the agar and incubated at 37ºC for 24 hours. All tests were performed in triplicate and zone of inhibition was measured after incubation with help of caliper. The antibiotic (tetracycline) disc and sterile water soaked disc served as positive and negative control, respectively.

Agar well assay method

Antimicrobial activity against all indicators microorganisms (starter culture and pathogenic/ spoilage organisms) was also carried out by agar well diffusion method reported by Deans *et al.* (1987) with slight modification. Molten tryptone glucose yeast agar containing 0.1 percent Tween 80 was seeded with 200 µL of indicator microorganism (10^5 CFU/mL) freshly grown for 18 hours poured into sterile Petri plates and the plates were allowed to solidify. Wells of 6mm diameter were made by using gel perforator followed by 100 µL of EO addition to respective wells. After incubation of plates at 37°C for 24 hours, zone of inhibition was noted down using slide calipers. Tetracycline solution of 1 mg/mL concentration and sterile water served as positive and negative control, respectively.

Minimal inhibitory concentrations (MIC) of EO against indicator microorganisms

Minimal inhibitory concentration of essential oil was assessed as the lowest concentration of oil inhibiting visible growth of the microorganism. The MIC is obtained by spiking selective growth media with varied concentrations of essential oil, followed by inoculation with indicator microorganisms. Different concentration of *Ocimum sanctum* essential oil ranging from 0-1 µL/mL was prepared with an increment of 0.1 µL/ mL in BHI (Brain Heart Infusion), MRS and M-17 broth. Indicator microorganisms (10^5 CFU/mL) were separately spiked to all respective broth having different concentration of EO in duplicate. All the tubes were incubated at 37°C for 24 hours. Survival of microorganisms was determined by spreading 100 µL of sample on Petri plate containing their selective medium as shown in Table I. After incubation for 37°C for 24 hours, growth on Petri plate was recorded. Growth of different indicator strains was also assessed through examination of turbidity relative to their controls. After initial inoculation, optical density (OD) of all controls was 0.450 and treated as baseline (OD~0.000) for comparison of OD obtained after 24 hours (Table. 3 and 4).

Compatibility of *O. sanctum* essential oil with Dahi and Yoghurt starters

250 mL standardized milk (3.5 % fat and 13 % SNF) was supplemented with 0, 0.5, 1.0, 1.5 and 2 µL/mL concentration of *O. sanctum* essential oil separately. The essential oil spiked milk was inoculated with yoghurt (NCDC-260) and Dahi (NCDC-167) cultures at the rate of 1 and 2 percent (10^8 CFU/mL). Contents were properly mixed and incubated at 42°C and 37°C respectively. Samples were assessed for curd setting time, pH and titratable acidity (TA) in terms of lactic acid (LA) produced (Table. 5).

Statistical analysis

The results were expressed as mean ± standard deviation. Significance was tested by employing analysis of variance (ANOVA). For computation of data software applications programs like MS Excel was used.

RESULTS AND DISCUSSION

Antimicrobial activity of essential oil

The observation on antimicrobial activity of *O. sanctum* EO against indicators strains in the present study have been furnished in Table. 2. The results on antimicrobial activity reveals maximum inhibition in terms of zone diameter of *S. aureus* NCDC 110 (29.2 ± 0.5 mm) and *B. cereus* ATCC 13061 (28.8 ± 0.4 mm) followed by *L. monocytogenes* ATCC 15303 (26 ± 0.4 mm), *E. coli* ATCC 25922 (24 ± 0.6 mm) and *S. typhi* NCDC 113 (21.5 ± 0.9 mm). Almost similar patterns were recorded with agar well assay method in relation to zone diameter of inhibition which was almost double to inhibition zone observed through disc diffusion method. The starter microorganisms showed much higher resistance to *O. sanctum* essential oil as compared to foodborne pathogens. The zone of inhibition (mm) in case of lactic acid bacteria was 6-8 mm in disc assay and 10-14 mm in agar well assay method. *Lactococcus lactis subsp. lactis* biovar. *diacetylactis* NCDC 60 was found most sensitive among starter microorganisms used as highest zone of inhibition of 14 ± 0.4 mm was resulted. These results are in agreement to disc assay observations reported by Da Silveira *et al*. (2012) with maximum inhibitory activity against *S. aureus*, prime suspect of mastitis milk contamination. Moreiraa *et al*. (2005) also reported disc assay results against different species of *E. coli* in tune with present study i.e. zone of inhibition 13 mm. Singh *et al*. (2005) also concluded, maximum antimicrobial activity of *O. sanctum* EO against *S. aureus* NCDC 110. Although there are very limited

studies regarding antimicrobial activity of essential oils (EOs) against starter cultures, they all reported lower susceptibility relative to foodborne pathogens (Saliu *et al.*, 2011; Upadhay *et al*., 2010; Thaweboon and Thaweboon, 2009) as depicted in present study. Antimicrobial activity action involves action of phenolic compounds which are found to have lesser effects against lactic acid bacteria (Rodriguez *et al.*, 2009).

Minimal inhibitory concentration of *O. sanctum* essential oil

MIC report of *O. sanctum* EO against indicator organisms revealed a large gap between inhibitory concentrations of pathogenic/spoilage microorganisms and dairy starters. Concentration ranges from 0.4-0.6 µL/mL was effective in restricting growth of pathogenic/spoilage microorganisms whereas minimum concentration of 1.8 µL/mL is required for bacteriostatic action against dairy starter culture (Table 3 and 4). The essential oil of *O. sanctum* has been effective against both Gram positive and Gram negative bacteria. Plating of samples from each concentration on their respective selective media resulted total inhibition of *S. aureus* NCDC 110 at concentration of 0.4 µL/mL followed by *B. cereus* ATCC 13061, *E. coli* ATCC 25922, *S. typhi* NCDC 113 and *L. monocytogenes* ATCC 15303 indicative of bactericidal concentration of 0.5µL/mL. Optical density measured at the above concentration also demonstrated same inhibitory trend by indicator strains. OD of 0.003 (OD of 0.450 is taken initial inoculated control and taken equivalent to OD~0.000 as described in material and methods) was observed for *S. aureus* NCDC 110 at 0.4 µL/mL after 24 hours of growth, which signifies nil

Table 1. List of selected indicator microorganisms and media used

S.No.	Pathogenic and spoilage microorganisms	Selective media used
1	*Bacillus cereus* ATCC 13061	*Bacillus cereus* agar base (BCAB)
2	*Staphylococcus aureus* NCDC 110	Baird Parker *Agar* Base (BPA)
3	*Listeria monocytogenes* ATCC 15313	Listeria Identification *Agar* Base (PALCAM)
4	*Salmonella typhi* NCDC 113	*Xylose- Lysine Deoxycholate Agar (XLD)*
5	*Escherichia coli* ATCC 25922	*Eosin Methylene Blue* Agar (EMB)
	Starter microorganisms	**Selective media used**
1	*Streptococcus thermophilus* NCDC 074	M-17 medium
2	*Lactobacillus bulgaricus* NCDC 009	(de Man, Rogosa and Sharpe) MRS medium
3	*Lactococcus lactis* NCDC 90	M-17 medium
4	*Lactococcus lactis subsp. lactis* biovar. *diacetylactis* NCDC 60	M-17 medium

Table 2. Antimicrobial activity of Ocimum essential oil by disc diffusion and agar well assay Method

Target Microorganism	Agar well assay inhibition zone diameter (mm)			Disc assay inhibition zone diameter (mm)		
	EO	Positive control	Negative control	EO	Positive control	Negative control
Bacillus cereus ATCC 13061	28.8 ± 0.4	24 ± 0.4	0	15 ± 0.9	22 ± 0.2	0
Escherichia coli ATCC 25922	24 ± 0.6	22 ± 0.2	0	13 ± 0.4	16 ± 0.2	0
Salmonella typhi NCDC 113	21.5 ± 0.9	26 ± 0.2	0	12 ± 0.3	18 ± 0.3	0
Staphylococcus aureus NCDC 110	29.2 ± 0.5	18 ± 0.4	0	14.5 ± 0.4	21 ± 0.4	0
Listeria monocytogenes ATCC 15313	26 ± 0.4	24 ± 0.3	0	12 ± 0.2	16 ± 0.2	0
Streptococcus thermophilus NCDC 074	12 ± 0.4	25 ± 0.4	0	7 ± 0.4	18 ± 0.2	0
Lactobacillus bulgaricus NCDC 304	10 ± 0.4	26 ± 0.4	0	6 ± 0.4	21 ± 0.3	0
Lactococcus lactis NCDC 090	11 ± 0.4	22 ± 0.2	0	6 ± 0.4	19 ± 0.4	0
Lactococcus lactis subsp. lactis biovar. *diacetylactis* NCDC 060	14 ± 0.4	23 ± 0.3	0	8 ± 0.4	20 ± 0.2	0

*****Mean ± standard deviation (SD) where n=4

Table 3. Visible growth of all target microorganisms at different concentration of essential oil

Essential oil concentration (µL/mL)	Target microorganisms: Pathogenic /spoilage microorganisms					Starter cultures			
	B. cereus ATCC 13061	*E. coli* ATCC 25922	*S.typhi* NCDC 113	*S. aureus* NCDC 110	*L.monocytogenes* ATCC 15313	*S.thermophilu s* NCDC 074	*L.bulgaricu s* NCDC 009	*L.lactis s* NCDC 090	*L. diacetylacti* NCDC 060
0.0	+	+	+	+	+	+	+	+	+
0.1	+	+	+	+	+	+	+	+	+
0.2	+	+	+	+	+	+	+	+	+
0.3	+	+	+	+	+	+	+	+	+
0.4	+	+	+	NG	+	+	+	+	+
0.5	NG	NG	NG	NG	NG	+	+	+	+
0.6	NG	NG	NG	NG	NG	+	+	+	+
0.7	NG	NG	NG	NG	NG	+	+	+	+
0.8	NG	NG	NG	NG	NG	+	+	+	+
0.9	NG	NG	NG	NG	NG	+	+	+	+
1.0	NG	NG	NG	NG	NG	+	+	+	+
1.2	NG	NG	NG	NG	NG	+	+	+	+
1.5	NG	NG	NG	NG	NG	+	+	+	NG
1.8	NG	NG	NG	NG	NG	NG	NG	NG	NG
2.0	NG	NG	NG	NG	NG	NG	NG	NG	NG

Response: (+) positive cell growth, NG - no measurable cell growth.

Table 4. Optical density of all target microorganisms at different concentration of essential oil

Essential oil concentration (µL/mL)	Target microorganisms								
	B. cereus ATCC 13061	*S. aureus* NCDC 110	*S.typhi* NCDC 113	*L.monocytogenes* ATCC 15313	*E.coli* ATCC 25922	*S.thermophilus* NCDC 074	*L.bulgaricus* NCDC 009	*L.lactis* NCDC 090	*L. diacetylactis* NCDC 060
Optical density after 4 h of treatment									
0.0	0.322±0.008	0.148±0.018	0.337±0.022	0.267±0.007	0.254±0.062	0.540±0.002	0.590±0.005	0.627±0.012	0.632±0.008
0.1	0.232±0.004	0.099±0.009	0.270±0.006	0.145±0.008	0.169±0.007	0.494±0.004	0.536±0.004	0.510±0.008	0.544±0.004
0.2	0.185±0.003	0.031±0.005	0.231±0.005	0.085±0.008	0.125±0.012	0.461±0.013	0.514±0.018	0.462±0.012	0.486±0.018
0.3	0.107±0.013	0.012±0.009	0.170±0.006	0.042±0.009	0.085±0.012	0.398±0.004	0.491±0.013	0.424±0.003	0.426±0.008
0.4	0.058±0.004	0.003±0.003	0.123±0.016	0.017±0.007	0.040±0.002	0.355±0.005	0.482±0.005	0.396±0.012	0.364±0.004
0.5	0.021±0.003	0.001±0.001	0.009±0.004	0.005±0.002	0.021±0.008	0.292±0.002	0.425±0.004	0.358±0.008	0.326±0.003
0.6	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.238±0.018	0.415±0.008	0.298±0.018	0.270±0.002
1.0	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.152±0.004	0.282±0.005	0.200±0.004	0.112±0.003
1.2	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.126±0.003	0.220±0.008	0.160±0.001	0.078±0.004
1.5	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.114±0.004	0.130±0.004	0.131±0.004	0.012±0.005
1.8	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.002±0.018	0.024±0.005	0.010±0.012	0.005±0.002
2.0	0.004±0.005	0.002±0.002	0.006±0.006	0.004±0.003	0.010±0.002	0.005±0.018	0.007±0.008	0.003±0.013	0.002±0.004

growth after inoculation while *S. typhi* NCDC 113 and *L. monocytogenes* ATCC 15303 at 0.5 µL/mL and *B. cereus* ATCC 13061 with *E. coli* ATCC 25922 at 0.6 µL/mL concentrations. Inhibitory concentration for dairy starters was found at 1.5 µL/mL for *Lactococcus lactis subsp. lactis* biovar. *diacetylactis* following *L. lactis*, *S. thermophilus* and *L. bulgaricus* at 1.8 µL/mL of concentration. These dairy starter cultures also fail to grow at above concentration when plated on M-17 and MRS agar after 24 hours of incubation. De Pasqua *et al*. (2005) also observed higher MIC values for *Lactobacillus* and *Lactococcus* dairy cultures as they are less susceptible as compared to pathogens and spoilage microorganisms like, *S. typhi, E. coli, S. aureus,* and *L. monocytogenes.*

Compatibility of dairy cultures with *O. sanctum* essential oil

The compatibility of dairy starters with varying concentration of *O. sanctum* (0.5, 1.0, 1.5 and 2.0 µL/mL) were determined in milk system by measuring change in pH, TA and curd setting time. The results on these aspects have been presented in Table 5. The observations showed that EO have no bacteriostatic effect on starters upto 1.0 µL/mL while an increase in concentration of essential oil (< 1.0 µL/mL) leads to decrease in viability of starter cultures. The curd setting time of 6 hours was observed at 1.5 µL/mL of essential oil at 1 and 2% starter inoculum for yoghurt while at this concentration there is loss of starter activity for dahi preparation. Although, this observation signifies greater sensitivity of dahi starters as compared to yoghurt cultures and an increased starter concentration upto 2% didn't prove worthier in both cases of yoghurt and dahi preparation as both concentrations resulted about similar lower physico-chemical properties. Again lower TA development for both the products 2 µL/mL represent no lactic acid formation or loss of starter culture activity but there is no effect on pH and TA characters of both products on 0.5 and 1.0 µL/mL concentration. General optimum setting time of 5 and 10 hours was recorded upto 1.0 µL/mL concentration for both products. Collectively, it also clarifies that greater concentration of EO (<1.0 µL/mL) is required for killing action towards lactic acid bacteria.

Singh *et al*. (2005) also reported decrease in pH in presence of essential oils but found concentration of 1.0 g/L of anise oil was not strong enough to down regulate yoghurt bacterial count below 10^7 CFU/mL during 10 days storage period alongwith titratable acidity of 1% LA. Similarly, during 28 days shelf life study, no significant difference was observed in *Lactobacillus acidophilus* viable counts among different samples containing variable concentration of essential oil

Table 5. Physico-chemical parameters of dahi and yoghurt prepared in presence of different culture levels and essential oil

Culture Levels (%v/v), 10^8 CFU/mL	Quality Attributes	Essential oil Concentration (µl/ml)				
		Control (0)	0.5	1	1.5	2
Yoghurt Culture						
1	pH	4.54±0.04^a	4.51±0.02^a	4.22±0.04^b	6.06±0.01^c	6.00±0.02^d
	TA (%LA)	0.84±0.01^a	0.88±0.01^a	0.98±0.04^c	0.78±0.02^d	0.62±0.04^e
	CST (h)	5.5	5.0	5	NCF	NCF
2	pH	4.5±0.03^a	4.56±0.02^a	4.14±0.04^b	5.95±0.03^c	5.78±0.02^d
	TA (%LA)	0.88±0.02^a	0.92±0.01^b	1.02±0.04^c	0.80±0.02^d	0.66±0.02^e
	CST (h)	4.5	4.5	6	8	NCF
Dahi Culture						
1	pH	4.73±0.02^a	4.84±0.01^b	4.34±0.03^c	6.21±0.02^d	5.63±0.04^e
	TA (%LA)	0.79±0.04^a	0.92±0.01^b	0.94±0.01^b	0.60±0.02^c	0.51±0.03^d
	CST (h)	9	9.5	10	NCF	NCF
2	pH	4.64±0.01^a	4.82±0.03^b	4.60±0.04^a	6.07±0.05^c	5.39±0.04^d
	TA (%LA)	0.90±0.04^a	0.96±0.01^b	0.94±0.01^{ab}	0.62±0.02^c	0.47±0.03^d
	CST (h)	9	10	10	NCF	NCF

*^{abcde} Mean values with different superscripts within a row differ significantly (P<0.01)
TA- Titratable acidity (%LA) ; CST- Curd setting time (h) ; NCF- No curd formation

of *Ziziphora clinopodioides* and *Mentha piperita* (Sarabi –Jamab and Niazmand, 2009).

CONCLUSION

This study demonstrated higher susceptibility of foodborne pathogens or spoilage organisms relative to dairy starters towards *O. sanctum* (Tulsi) essential oil. Antimicrobial trials involving agar well and disc assay methods depicts that *O. sanctum* EO is more efficacious against Gram negative bacteria than Gram positive bacteria. These results also have shown that concentration of 0.5-1.0 µL/mL can be directly used for preservation purpose without affecting starter cultures activity vis-à-vis quality in fermented products. The EO doesn't affect the products physico-chemical attributes and curd setting time at concentration of 0.5-1.0 µL/mL however these concentration completely inhibited pathogens and spoilage microorganisms. Therefore, use of lactic starters and *O. sanctum* (Tulsi) essential oil as natural preservative can provide dual benefits i.e. health promotion as well as enhanced food safety and shelf life.

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