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Thyme *Thymus vulgaris* L. Thymol CT Essential Oil as Natural Preservative

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Abstract
There is growing interest in natural ingredients that can be used as preservatives in the food industry. Significant attention is dedicated to essential oils as they often exhibit antioxidant and antimicrobial properties. *Thymus vulgaris* L. essential oil is considered to be particularly promising based on the preliminary studies. Still, significant disagreement exists between the reported data. This disagreement can be explained by the presence of different chemotypes of *T. vulgaris* essential oil.
In this article, I analyze recent research focusing on the thymol chemotype (CT) of *T. vulgaris* (>40% thymol). Reviewed studies demonstrate that this essential oil exhibits a broad scale antimicrobial activity against foodborne pathogens. When used with bread and dairy products, it can improve food preservation without degrading the sensory perception of a product. Results demonstrate that *T. vulgaris* Thymol CT essential oil has a very strong potential applicability as an antimicrobial agent in the food industry.

Keywords: *Thymus vulgaris* L., essential oil, thymol, preservative

1. Introduction
There is significant interest in the use of natural ingredients as preservatives in the food industry. Essential oils are particularly appealing candidates as they often possess both antimicrobial and antioxidant properties. Promising results were reported for oregano *Origanum vulgare* L. essential oil. For example, an addition of small amount of oregano essential oil (up to 1% v/w) to ground meat delayed microbial growth [1], while its addition to beef meat fillets contaminated with *Listeria monocytogenes* significantly reduced bacterial population [2]. Citrus essential oils were also reported to suppress the growth of many bacteria causing food poisoning [3, 4] and acted as effective antimicrobial agents when added to dairy and bread products [5, 6].
In order to replace synthetic preservatives, essential oils need to meet a few criteria including low cost and high effectiveness. Essential oil of thyme *Thymus vulgaris* L. is a viable candidate based on the preliminary reports of good radical scavenging, antibacterial, and antifungal activities. This herb, originally native to southern Europe, is now cultivated all over the world; the essential oil is widely available and has a relatively low cost.
Still, there is some variability in the reported data that can be due to the presence of different chemotypes (CTs). Recent study of published *T. vulgaris* essential oil compositions identified 20 different CTs including Borneol CT, Carvacrol CT, Geraniol CT, Linalool CT, Thymol CT, Camphor/Camphene CT, 1,8-Cineole/Alpha-Terpinyl Acetate CT, Cyclocitral/Verbenol CT, Geranyl Formate/Geraniol CT, Para-Cymene /Thymol CT, Alpha-Terpinyl Acetate/Carvacrol CT, Sabinene Hydrate/Terpinene-4-ol CT, and other complex CTs with more than two dominant constituents [7]. Significant variability was reported even for the samples of *T. vulgaris* originating from the same geographic region. For example, seven CTs were reported for essential oil from Southern France: Borneol CT [borneol (34%), camphene (9%), alpha-terpineol (9%), thymol (7%), alpha-pinene (6%), para-cymene (6%), and methyl-thymol (6%)], Carvacrol CT [carvacrol (42%), para-cymene (27%), gamma-terpinene (11%), and thymol (5%)], Para-Cymene CT [para-cymene (32%), carvacrol (21%), thymol (11%), gamma-terpinene (9%)], Geraniol CT [geranyl formate (48%), geraniol (26%), and beta-caryphyllene (8%)], Linalool CT [linalool (78%), beta-caryphyllene (8%), and linalyl acetate (7%)], Sabinene hydrate/Terpinene-4-ol CT [sabinene hydrate (30%), terpinene-4-ol (21%), beta-caryphyllene (4%)], and Thymol CT [thymol (63%) and para-cymene (19%)] [8].
Variations in the chemical composition will result in different antioxidant and antibacterial properties.

Karami-Osboo *et al*., 2010 [9] used solvent separation to determine active constituents in *T. vulgaris* essential oil [thymol (50%), para-cymene (20%), carvacrol (8%), trans-caryophyllene (7%)]. When tested against *Erwinia amylovora*, phenols (carvacrol and thymol) were identified as the antibacterial agents. When individual constituents of *T. vulgaris* essential oil were tested against strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, thymol always caused inhibition, linalool was not effective against *P. aeruginosa* and *E. coli*, carvacrol was not effective against *P. aeruginosa*, and linalyl acetate did not cause any inhibition [10]. Thus, thymol content is believed to be particularly important for the antimicrobial action.

Mechanism of action of *Thymus* spp. [*T. eriocalyx* (64% thymol) and *T. x-porlock* (32% thymol)] essential oils against food pathogenic bacteria (*Listeria monocytogenes*) was also investigated. Treatment with essential oils caused roughening, thickening, and eventual rupture of the cell walls. Cells lacked cytoplasm due to the decrease of the cell membrane functionality as a barrier [11]. This can explain significant variability in antimicrobial properties of individual essential oil constituents. Not only the presence of specific functional groups but also their location (as in the case of thymol and carvacrol), will affect the ability of constituents to penetrate the microbial cell membrane and cause alterations in its structure and functionality.

The purpose of this article is to assess the applicability of *T. vulgaris* Thymol CT essential oil as a preservative agent. To assure consistency, only studies with reported thymol concentration of at least 40% are reviewed.

2. Results and Discussion

We identified eight publications that evaluated the antibacterial and antifungal potential of *T. vulgaris* Thymol CT essential oil with the defined composition. Agar disk-diffusion, agar well-diffusion, and broth dilution methods were utilized in the studies.

Antimicrobial activity of eight essential oils, including *T. vulgaris* [thymol (79%), carvacrol (5%)] was studied by Sartoratto *et al*., 2010 [12]. *Thymus vulgaris* essential oil showed a strong activity against *Bacillus subtilis* and *Enterococcus faecium* (minimum inhibitory concentration (MIC) of 0.15 – 0.50 mg/mL), moderate activity against *Salmonella choleraesuis* and *S. aureus* (MIC of 0.6 – 1.0 mg/mL), and weak activity against *Candida albicans*, *Micrrococcus luteus*, and *Rhodococcus equi* (MIC of 2.0 mg/mL or larger).

Teixeira *et al*., 2013 [13] assessed the effect of *T. vulgaris* essential oil on the growth of seven food-borne spoilage and pathogenic bacterial strains (*Brochothrix thermosphacta*, *E. coli*, *Listeria innocua*, *L. monocytogenes*, *Pseudomonas putida*, *Salmonella typhimurium*, and *Shewanella putrefaciens*). Essential oil inhibited the growth of all bacteria tested with the highest reduction achieved for *L. innocua* and *L. monocytogenes*.

Table 1: Bacterial growth inhibitory data for *Thymus vulgaris* L. essential oil obtained via disk diffusion method. Inhibition zone radius is measured by using 20 µL of undiluted essential oil. Effect of minimum inhibitory concentration is presented as mean bacterial Colony Forming Units logarithmic reduction per mL of essential oil. Data are adopted from Teixeira *et al*., 2013 [13].

Essential Oil Main Constituents	Method	Bacterial Strains	Inhibition zone radius (mm)	Effect of minimum inhibitory concentration (CFU/mL)
Thymol (75%), carvacrol (5%), para-cymene (4%)	Disk Diffusion	*B. thermosphacta*	32	4.5
		E. coli	47	2.9
		L. innocua	44	8.0
		L. monocytogenes	45	8.0
		P. putida	40	2.5
		S. typhimurium	57	7.2
		S. putrefaciens	32	Not reported

A study conducted by Stojković *et al*., 2013 [14] also demonstrated that *T. vulgaris* essential oil [thymol (49%), para-cymene (19%)] was active against *S. aureus* and *S. typhimurium* with MIC of 1.0 µL/ml. In addition, synergistic interaction with *O. vulgare* essential oil [carvacrol (65%), gamma-terpinene (11%), para-cymene (11%)] was observed. This suggests the possibility that complex CTs, like Carvacrol/Gamma-Terpinene/Thymol, have a stronger antimicrobial action than Thymol CT.

Results were further supported by the study conducted by Valizadeh *et al*., 2016 [15]. *T. vulgaris* essential oil [thymol (64%), gamma-terpinene (9%), para-cymene (6%)] inhibited the growth of four bacterial strains (*Bacillus cereus*, *L. monocytogenes*, *E. coli*, and *S. typhimurium*) with the strongest activity against *B. cereus*. It also had a strong inhibitory effect on fungal strains of *Candida spp*. (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. dubliniensis*).

T. vulgaris essential oil [thymol (44%), para-cymene (20%), carvacrol (11%), thymoquinone (6%)] was successfully tested as an antibacterial agent in cheese industry [16]. The addition of essential oil to feta cheese inoculated with *E. coli* O157:H7 or *L. monocytogenes* significantly reduced the survival time of bacteria. *E. coli* O157:H7 and *L. monocytogenes* strains survived up to 32 and 28 days, respectively, in control samples. The addition of *T. vulgaris* essential oil reduced the survival time in a dose-dependent manner to 22 and 18 days (0.1 ml/100 g) and 16 and 14 days (0.2 ml/100 g), respectively. Importantly, feta cheese samples with essential oil added successfully passed sensory evaluation conducted by the panelists. Thus, *T. vulgaris* essential oil can be used as a preservative in cheese without degrading the sensory perception of a product.

Thymus vulgaris essential oil [thymol (71%), alpha-terpinene (7%)] was also shown to be useful as antifungal and aflatoxin (AF) inhibiting chemical. It both suppressed *Aspergillus parasiticus* growth and inhibited AFs production. The median inhibitory concentrations (IC_{50}) were 93.5 µg/ ml and 11.7 µg/ ml for aflatoxin B_1 and aflatoxin G_1, respectively [17].

Antifungal potential of thyme essential oil attracted the attention of the agricultural industry with essential oil being considered for the control of postharvest pathogens. *Thymus vulgaris* essential oil [thymol (25-50%), para-cymene (20-25%), gamma-terpinene (5%-10%), linalool (5%-10%)] suppressed the growth of *Botrytis cinerea*, an important postharvest pathogen. Application of thyme essential oil to strawberry fruits inoculated with the *B. cinerea* significantly delayed the appearance of fungal infection. The signs became visible on day four after inoculation in untreated fruits, day five and day eight when 200 µl/ml and 500 µl/ml solutions were used [18].

Microencapsulation was reported as an effective way to use thyme essential oil as an antimicrobial agent. *Thymus vulgaris* essential oil [thymol (54%), para-cymene (16%), carvacrol (7%)] inhibited growth of seven Gram-positve and Gram-negative bacterial strains (*E. faecium*, *Enterococcus hirae*, *S. aureus*, *E. coli*, *Salmonella chloreaesuis*, *S. typhimurium* and *P. aeruginosa*) with the strongest activity against *S. aureus* and the weakest activity against *E. faecium* [MIC varied between 0.125 mg/ml and 0.60 mg/ml]. It was also effective against two fungal strains (*C. albicans* and *Aspergillus niger*) with the MIC of 0.25 mg/ml. Microencapsulation of *T. vulgaris* essential oil reduced MICs nearly ten times, to 0.01 - 0.03 mg/ml level. Application of spray containing essential oil microparticles to the surface of cake samples delayed spoilage and increased the shelf life from less than 15 days to an excess of 30 days [19].

Still, additional research is necessary to fully understand the interplay between the composition and the antimicrobial action of essential oil. Nikolić *et al*., 2014 [20] assessed the antimicrobial activity of *Thymus spp*. essential oils with high thymol content. *Thymus serpyllum* [thymol (39%), para-cymene (9%), gamma-terpinene (7%), borneol (6%), and carvacrol (5%)], *T. algeriensis* [thymol (56%), carvacrol (14%), para-cymene (6%), gamma-terpinene (5%)], and *T. vulgaris*. [thymol (49%), para-cymene (20%), gamma-terpinene (4%), carvacrol (4%)] essential oils were tested against eight bacterial strains (*Enterococcus faecalis*, *Lactobacillus acidophilus*, *P. aeruginosa*, and *Staphylococcus spp.*) and *Candida spp*. All three essential oils exhibited significant activity against the tested strains; *T. serpyllum* essential oil was the most potent and *T. vulgaris* exhibited the lowest antimicrobial effect. Observed behavior does not align with expected based solely on the phenol content (highest for *T. algeriensis* and lowest for *T. serpyllum*) and suggests that minor constituents should be taken into account as well to fully understand the antimicrobial action.

3. Conclusions

Thymus vulgaris Thymol CT (>40% of thymol) essential oil inhibited growth of a number of food-borne spoilage and pathogenic bacterial strains including *Bacillus spp.*, *Brochothrix thermosphacta*, *Enterococcus spp.*, *Erwinia amylovora*, *Escherichia coli*, *Listeria spp.*, *Micrrococcus luteus*, *Pseudomonas putida*, *Rhodococcus equi*, *Salmonella spp.*, *Shewanella putrefaciens*, and *Staphylococcus spp*. It was effective against fungal strains of *Candida spp*. and *Aspergillus spp*. The MIC values varied between 0.1 mg/ml and 2 mg/ml. The essential oil was successfully used as an antibacterial agent in bread and cheese industries and applied for postharvest treatment of fresh strawberries. Microencapsulation significantly enhanced the activity of essential oil and reduced MIC values nearly ten times.

Synergistic interaction between *T. vulgaris* and *O. vulgare* essential oils was reported. In addition, the study of the antimicrobial action of *Thymus spp*. essential oils suggested that antimicrobial activity could not be deducted directly from the phenol content.

These results demonstrate significant potential for the use of *T. vulgaris* Thymol CT essential oil as an antimicrobial in the food industry. They also highlight the importance of further research on the relationship between the composition and antimicrobial properties.

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