# The Use of Essential Oils in Ruminants as Modifiers of Rumen Microbial Fermentation

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## 0. Take Home Message

The efficiency of energy and protein utilization in the rumen may be improved through the manipulation of microbial population. Antibiotic ionophores have been very successful in improving these efficiencies, but its use in animal feeds is facing reduced social acceptance. For this reason, other alternatives to control specific microbial populations are currently being investigated. Some essential oils have antimicrobial activities resulting in reduced peptidolysis, deamination and methanogenesis. Because the effects of essential oils are diet and pH dependent, the selection of the appropriate essential oil will depend on the specific objectives and target animal. Moreover, because essential oils may act at different levels in the energy and protein metabolic pathways, their careful selection and combination may provide a useful tool to effectively manipulate rumen microbial fermentation.

## 1. Introduction

The efficiency of energy and protein utilization in the rumen is relatively low and can be improved by the modulation of several metabolic pathways, including the inhibition of methane production and deamination. This low efficiency not only reduces production performance, but also contributes to the release of pollutants to the environment (Tamminga, 1996). This efficiency can be improved by modulating the activity of specific rumen microbial populations involved in the previously mentioned metabolic pathways. For example, the utilization of ionophores, a potent antibiotic against gram-positive bacteria, has proven effective in improving the efficiency of energy and protein utilization in the rumen (Van Nevel and Demeyer, 1988). However, the use of antibiotics in animal feeds is facing reduced social acceptance due to the appearance of residues and resistant strains of bacteria, and the search for alternatives, including yeast, organic acids, plant extracts, probiotics and antibodies, has become necessary (Calsamiglia et al., 2006).

Plants produce an extensive variety of organic compounds derived from their secondary metabolism that are classified in three main groups: saponins, tannins, and essential oils. Some essential oils have antimicrobial activities and are currently considered safe for human and animal consumption, and are categorized as GRAS (Generally Recognized as Safe; FDA, 2004) in the USA. Their potential use in ruminants has been recently reviewed by Calsamiglia et al. (2007) and Benchaar et al. (2007).

The objective of the present paper is to review current knowledge on the potential benefits of essential oils and their active components as modifiers of rumen microbial fermentation.

## 2. Origin, Clasification and Activity of Essential Oils

Essential oils are a blend of secondary metabolites obtained from the plant volatile fraction by steam distillation (Gershenzon and Croteau, 1991). They are generally clasified in two chemical groups that originate from different precursors of the primary metabolism and are synthesized through separated metabolic pathways (Figure 1 and 2):

a. Terpenoids: Terpenoids are the more numerous and diversified group of plant secondary

metabolites and derive from an isoprenoid structure ($C_5H_8$). Within terpenoids, the most important components of essential oils belong to the monoterpenoid and sesquiterpenoid families (Gershenzon and Croteau, 1991).

b. Phenylpropanoids. They are not the most common compounds of essential oils and derive from a structre with a chain of three carbons bound to an aromatic ring of 6 carbons.

Essential oils have a wide variety of effects on health, including positive effects on cardiovascular diseases, some tumors, inflammatory processes and, in general, diseases in which the uncontrolled proliferation of free radicals is very damaging (Harborne and Williams, 2000; Reddy et al., 2003; Trouillas et al., 2003). However, the most important activity of these compounds is as antiseptics and antimicrobials (Burt, 2004; Table 1).

Terpenoids and phenylpropanoids develop their action against bacteria through the interaction with the cell membrane (Griffin et al., 1999; Davidson and Naidu, 2000; Dorman and Deans, 2000). This interaction causes conformational changes in the membrane and results in the leakage of ions across the cell membrane and the loss of the transmembrane ionic gradient (Griffin et al., 1999). In most cases, large amounts of energy are diverted to this function and bacterial growth is reduced and, in some cases, microbial death occurs (Griffin et al., 1999; Ultee et al., 1999; Cox et al., 2001). This mechanism of action makes these essential oils more effective against gram-positive bacteria, where the cell membrane can interact directly with hydrophobic compounds of essential oils (Smith-Palmer et al., 1998; Chao and Young, 2000; Cimanga et al., 2002). However, and in contrast to monensin and other ionophores, the small molecular weight of most essential oils allows them to cross the external hydrophilic cell wall of gram-negative bacteria, being also active against them (Calsamiglia et al., 2007; Griffin et al., 1999; Dorman and Deans, 2000). This property of being active against gram-positive and negative bacteria reduces the selectivity of these compounds against specific populations, making more difficult the modulation of rumen microbial fermentation. Other mechanisms of action have also been described, including the coagulation of some cell constituents, denaturation of proteins and interaction with DNA (Gustafson et al., 1997; Juven et al., 1994). The essential oil of garlic is a special case because many compounds of this oil are not in the whole plant, but are produced from thiosulfates during the steam treatment of the plant (Pentz and Siegers, 1996). The main antimicrobial mechanism appears to be related to its capacity to interact with the sulphydril groups (-SH) of other active compounds (Reuter et al., 1996), resulting also in instability of the cell membrane of some specific microbial groups (see discussion on garlic below).

## 3. Effects of Essential Oils on Ruminal Fermentation

The potential use of essential oils in ruminant diets was first reported in the '60s (Borchers, 1965; Oh et al., 1967, 1968; Nagy and Tendergy, 1968). However, as a result of the approval of the use of antibiotics in animal feeds at the beginning of the '70s, few studies were published thereafter (Broderick and Balthrop, 1979). Only after the announcement of the ban on the use of antibiotics as feed additives in the EU, has there been a renewed interest in the study of essential oils as rumen microbial modifiers. Since then, more than 20 different studies have been published. In most cases, a positive effect was described as an increase in propionate and decrease in acetate, methane and ammonia-N production without reducing total VFA production. The effect of the main essential oils available to be used as feed additives will be presented separately by active components.

### *3.1. Carvacrol and thymol*

Carvacrol and thymol are monoterpenoids with a strong antimicrobial activity against a wide range of gram-positive and negative bacteria. Both are found in oregano (*Origanum* spp.) and thyme

(*Thymus* ssp.). Busquet et al. (2005a) reported that in vitro, carvacrol (2.2 mg/L) decreased large peptide and increased ammonia-N concentrations at 2 h after feeding, suggesting that it either inhibited proteolysis or stimulated peptide lyses. Higher doses (300 mg/L) increased pH and butyrate proportion, and decreased acetate and propionate proportions, and total VFA concentration.

Brochers (1965) was the first to report that thymol inhibited deamination. A similar conclusion was reached by Broderick and Balthrop (1979) after incubating rumen fluid with thymol in vitro. More recently, Evans and Martin (2000) reported that thymol affected energy metabolism of two relevant rumen bacteria grown in pure culture: *Streptococcus bovis* and *Selenomonas ruminantium*. It reduced methane and lactate concentrations, although at higher doses also reduced overall nutrient digestion and total VFA production, a clear indication that microbial metabolism was inhibited. However, moderate doses resulted in an increase in the acetate to propionate ratio. It is interesting to point out that *S. ruminantium* was more sensitive to thymol than *S. bovis* which may result in an increased accumulation of lactic acid because the production of lactate by *S. bovis* (a major lactic acid producer) may not be metabolized fast enough by *S. ruminantium* (a major lactic acid utilizer). Castillejos et al. (2006) reported that low doses of thymol (50 mg/L) had no effects on in vitro rumen microbial fermentation, but at higher doses (500 mg/L) total VFA and ammonia-N concentrations decreased, and the acetate to propionate ratio increased. Similar results were reported in a long-term continuous culture fermentation study. Furthermore, several in vitro studies suggested that the effects of thymol are diet and pH dependent (Castillejos et al., 2006; Cardozo et al., 2005). While Castillejos et al. (2006) reported that thymol increased the acetate to propionate ratio in 60:40 alfalfa hay:concentrate diets at high pH (6.4), Cardozo et al. (2005) observed changes in the opposite direction (a reduction in the acetate to propionate ratio) when thymol was incubated in rumen fluid from cattle fed a 10:90 straw:concentrate (based on corn, barley and soybean meal) diet at pH 5.5. Therefore, it is important to define the conditions where these additives are used to modify rumen microbial fermentation in the desired direction. Compounds with phenolic structures, such as thymol and carvacrol, are more effective as antimicrobials in comparison with other non-phenolic secondary plant metabolites due to the presence of a hydroxyl group in the phenolic structure (Helander et al., 1998; Ultee et al., 2002). Furthermore, the small molecular weight of thymol and carvacrol allows them to gain access to the cell membrane through the pores of the external wall. As a result of the strong and wide-spectrum activity against gram-positive and negative bacteria, the narrow margin of security between an optimal and a toxic dose, and the effects reported, which were not always in the desired direction (Castillejos et al., 2006), suggest that their antimicrobial activity may be too strong and non-specific to modulate the fermentation in a complex microbial environment such as the rumen.

### *3.2. Cinnamadehyde, eugenol and anethol*

Cinnamaldehyde, eugenol and anethol are phenylpropanoids with a wide spectrum of antimicrobial activity against gram-positive and negative bacteria.

Cinnamaldehyde is the main active component of cinnamon (*Cinnamomum cassia*) oil, accounting for up to 75% of its composition. Cardozo et al. (2004), in a continuous culture experiment, were the first to suggest that cinnamon oil modified N metabolism of rumen microorganisms by inhibiting peptidolysis, but the effects on VFA concentration was negligible. Higher doses of cinnamon oil and cinnamaldehyde decreased total VFA and ammonia-N concentrations, although cinnamaldehyde had stronger effects compared with cinnamon oil (Busquet et al., 2006). However, the effects on the proportions of individual VFA were different, and while cinnamon oil increased acetate without affecting propionate or butyrate molar proportions, cinnamaldehyde increased propionate without affecting acetate and butyrate

proportions. These results suggest that, although cinnamaldehyde is the main and most active component in cinnamon oil, other substances within cinnamon oil may interact with cinnamaldehyde, although cinnamaldehyde resulted in a more desirable fermentation profile. Busquet et al. (2005ab) tested the effect of low doses of cinnamaldehyde in a long-term, dual flow, continuous culture fermentation study and reported similar trends, where cinnamaldehyde numerically decreased the molar proportion of acetate and numerically increased the proportion of butyrate, but differences did not reach significance. When higher doses (31.2 and 312 mg/L) were used (Busquet et al., 2005b), cinnamaldehyde reduced the molar proportion of acetate and increased the molar proportions of propionate and butyrate. This fermentation profile is similar to that observed when antimethanogenic compounds, such as amicloral or carbon monoxide, were used to modify rumen microbial fermentation, suggesting that its mechanism of action may involve the inhibition of methanogenesis (Horton, 1980; Hino and Russell, 1985), but the hypothesis needs to be confirmed with experimental data. The effects of cinnamaldehyde on N metabolism were inconsistent. While some studies reported changes in N metabolism (Cardozo et al., 2004; Busquet et al., 2005a) others found no effects (Busquet et al., 2005b). At least part of these inconsistencies may be related to the dose used. However, Ferme et al. (2004) reported that the addition of cinnamaldehyde to an in vitro rumen simulation system resulted in a reduction in *Prevotella* spp., a group of bacteria known to be involved in deamination, providing evidence of a mechanism of action. Results presented in the previous discussion were conducted using rumen fluid from dairy cattle and a high forage diet, but as demonstrated in other essential oils, the effects may change depending on the type of diet and pH. Cardozo et al. (2005) tested the effects of cinnamon oil and cinnamaldehyde in vitro using rumen fluid from beef cattle fed a 10:90 forage to concentrate diet. At pH 7.0, cinnamon oil and cinnamaldehyde resulted in higher acetate to propionate ratio and lower total VFA concentration, suggesting a lower efficiency of nutrient utilization in the rumen. In contrast, at pH 5.5, total VFA increased and ammonia-N concentration and the acetate:propionate ratio decreased with cinnamon oil and cinnamaldehyde. Juven et al. (1994) already reported that the antimicrobial effect of cinnamon oil increased as pH decreased from 6.5 to 5.5. Results indicate that cinnamon oil and cinnamaldehyde have the potential to improve nutrient utilization in the rumen, but in beef production systems the effects may be more relevant in feeding conditions that favor low ruminal pH. Busquet et al (2003) reported that the addition of cinnamadehyde to lactating dairy cattle resulted in a slight reduction in feed intake (0.3 kg/d), but an increase of 1.0 L/d in milk production, although differences did not reach significance.

Eugenol is one of the main active components of clove bud (*Eugenia caryophyllus* or *Syzygium aromaticum*) and cinnamon (*Cinnamomum cassia*) oils (accounts for up to 85 and 8% of these oils, respectively; Davidson and Naidu, 2000). In a continuous culture study, low doses of clove bud oil resulted in lower molar proportions of acetate and branch-chained VFA (BCVFA) and higher molar proportion of propionate (Busquet et al., 2005a). Clove bud also affected N metabolism, increasing peptide N and numerically decreased AA-N concentrations, suggesting that it decreased the peptidolytic activity in the rumen. In an in vitro batch culture dose response study, Busquet et al. (2006) confirmed that clove bud oil affected rumen fermentation, reducing total VFA and ammonia-N concentrations, and showing a linear increase in the molar proportion of propionate, and a quadratic effect on the molar proportions of acetate and butyrate. In general terms, the effects of eugenol were similar to those reported for clove bud oil. The fermentation profile observed suggests that, when used at optimal doses, efficiency of energy and protein utilization in the rumen was improved. The potential benefits of eugenol on rumen microbial fermentation were further tested in vitro by Castillejos et al. (2006) with two different types of diets. Data confirmed that in a 60:40 forage:concentrate dairy cattle diet based on alfalfa hay, corn grain, barley grain and soybean

meal, eugenol reduced ammonia-N and BCVFA concentrations, suggesting that deamination was inhibited. Effects on VFA production and proportions were more variable. In contrast, in a 10:90 beef-type diet based on straw, corn grain, barley grain and soybean meal, eugenol reduced total VFA concentration and the proportion of propionate, and increased the proportion of acetate and the acetate to propionate ratio. This fermentation profile may not be desirable for beef production. Therefore, it appears that eugenol may improve VFA production and profile, and N utilization in the rumen of lactating animals, but the fermentation profile does not support its recommendation for beef cattle diets.

Anethol is the main active component of anise (*Pimpinella anisum*) oil and is responsible for its antimicrobial activity that has been related to the ether group on its aromatic ring (Figure 1 and 2; Davidson and Naidu, 2000). In vitro studies with rumen fluid showed that anethol and anise oil decreased total VFA and the proportions of acetate and propionate, and increased the proportion of butyrate, although anethol had stronger effects compared with anise oil. Anise oil and anethol did not affect ammonia-N concentration (Busquet et al., 2005a). In contrast, Cardozo et al. (2004) suggested that lower doses of anise oil in continuous culture stimulated protein degradation, increasing the concentration of peptides and ammonia-N, but reported no effects on the VFA profile. However, when anise oil was used in in vitro fermentation studies using rumen fluid from beef cattle fed a 10:90 forage to concentrate diet and pH 5.5, ammonia-N concentration and acetate proportion decreased, and propionate proportion increased without affecting total VFA production (Cardozo et al., 2005). Cardozo et al. (2006) supplemented anise oil to growing heifers fed a 10:90 forage to concentrate diet and reported a trend to increase DM intake. Total VFA concentration was not affected, but the molar proportion of acetate decreased and the molar proportion of propionate increased, and the acetate to propionate ratio decreased. Anise oil also decreased ammonia-N concentration and protozoa counts. These results are consistent with previous in vitro trials simulating beef cattle conditions fed high concentrate diets (Cardozo et al., 2005; Fandiño et al., 2006). Our results suggest that anise oil inhibited deamination of amino acids and reduced the acetate to propionate ratio in the rumen, and may be beneficial for beef production systems.

### *3.3. Capsaicin*

Capsaicin is tetraterpenoid found in hot peppers (*Capsicum annum* ssp.) and is the main component of capsicum oil (10 to 15%; Cichewicz and Thorpe, 1996). When supplied to in vitro culture of rumen fluid from dairy cattle, the effects have been negligible in the short and long term (Cardozo et al., 2004; Busquet et al., 2005a). However, Cardozo et al. (2005) demonstrated that the effects were different in an in vitro system with rumen fluid from beef cattle fed a 10:90 straw:concentrate ratio diet, and reported that at pH 7.0 total VFA and ammonia-N concentrations were reduced and the acetate to propionate ratio increased. In contrast, at pH 5.5 capsicum oil reduced ammonia-N concentration, increased total VFA production and propionate proportion, and reduced acetate proportion and the acetate to propionate ratio. Therefore, its effects on high concentrate diets at low pH suggest that nutrient utilization in the rumen may be improved. The low pH appears to shift the molecule to a more hydrophobic status, being more active as antimicrobial. Cardozo et al. (2006) tested the effect of feeding capsicum oil in ruminally cannulated beef cattle fed a high concentrate diet. Total VFA concentration was not affected, but the molar proportion of acetate decreased. Capsicum oil reduced the ruminal concentration of large peptides and increased that of small peptides and amino acids, but had no effect on ammonia-N concentrations. These effects suggest that it stimulated peptidolysis, which may provide more peptides and amino acids, and enhance microbial protein synthesis and flow to the small intestine. Although these changes were in the same direction as the in vitro study (Cardozo et al., 2005), effects were smaller. However, this effect was not always consistent

(Cardozo et al. 2004; Busquet et al., 2005a). In addition, capsicum oil increased DM and water intake. There is evidence that capsaicin increases DM and water intake in rats and humans (Zafra et al., 2003; Calixto et al., 2000). Similar results were reported by Fandiño et al. (2006) and Rodriguez et al. (unpublished results). Therefore, there seems to be potential for using capsaicin in beef cattle diets based on its effects increasing DM intake and potential effects on rumen microbial fermentation.

### *3.4. Garlic oil*

Garlic oil is a mix of a large number of different molecules that are found in the plant or are the result of changes occurring during oil extraction and processing (Lawson, 1996). Although garlic oil is known for its therapeutic properties (anti-parasitic, insecticidal, anti-cancer, anti-oxidant, immunomodulatory, anti-inflammatory, hypoglycemic), its antimicrobial activity against a wide spectrum of gram-positive and negative bacteria is its most prominent activity and has been thoroughly studied (Reuter et al., 1996). Busquet et al. (2005abc and 2006) have consistently shown that garlic oil reduced the proportions of acetate and BCVFA, and increased the proportions of propionate and butyrate. This fermentation profile is different from that of monensin (that reduces the acetate to propionate ratio and butyrate concentrations) and is consistent with changes observed when methane inhibitors are supplied to rumen microbes (Chalupa et al., 1980; Martin and Macy, 1985). In fact, in vitro studies demonstrated that garlic reduced the $CH_4$ (µmol):VFA (µmol) ratio from 0.20 to 0.05 (Busquet et al., 2005). Methane is the main hydrogen sink in the metabolic pathway of rumen fermentation, and the inhibition of its synthesis generates reducing equivalents that need to be disposed of, propionate and butyrate being the main alternatives (Van Nevel and Demeyer, 1988). In order to identify the main active component in garlic oil, the oil and four purified active components (allicin, diallyl sulfide, diallyl disulfide and allyl mercaptan) thought to play a major role in its antimicrobial activity were tested in vitro to determine their effect on rumen microbial fermentation (Busquet et al., 2005c). Garlic oil, diallyl disulphide and allyl mercaptan reduced acetate and methane, and increased propionate and butyrate proportions to the same extent, suggesting that diallyl disulphide and allyl mercaptan they were responsible for most of its effects. In contrast, allicin and diallyl sulfide had minor effects on rumen microbial fermentation. Similar results have also been reported recently by Kamel et al. (2007). The effects of garlic oil and its main active components on N metabolism were more variable. While Cardozo et al. (2004) suggested that garlic oil inhibited deamination, others reported only small and variable effects (Busquet et al., 2005bc). Ferme et al. (2004) reported that garlic modified the microbial population profile in a continuous culture experiment, reducing the contribution of *Prevotella* ssp. to the overall microbial population in the rumen. *Prevotella* ssp. are mainly responsible for protein degradation and amino acid deamination. Cardozo et al. (2005) also tested the effects of garlic oil using rumen fluid and a high concentrate diet typically found in feedlot beef at different pH (7.0 vs. 5.5). While at pH 7.0 garlic oil resulted in lower ammonia-N and total VFA concentrations, at pH 5.5 ammonia-N concentration was also reduced, but total VFA concentration and propionate proportion increased, and acetate proportion and the acetate to propionate ratio decreased compared with a control with no garlic, suggesting a shift in rumen microbial fermentation. As pH decreases, acids tend to become undissociated and more hydrophobic, therefore interacting more easily with cell membranes and exerting their antimicrobial effect. Furthermore, bacteria seem to be more susceptible to the effects of essential oils at low pH (Skandamis and Nychas, 2000).

The mechanism of action of garlic oil and its main active components on rumen microbial fermentation are very different from all other essential oils. Busquet et al. (2005bc) suggested that the anti-methanogenic effect of garlic and its active components was the result of the direct inhibition of *Archaea* microorganisms in the rumen through

the inhibition of HMG-CoA reductase (Gebhardt and Beck, 1996), and as a result, the reduction in the synthesis of the isoprenoid unit responsible for the cell membrane stability. Miller and Wolin (2001) provided further support for this hypothesis by demonstrating that lovastatin and mevastatin, which decrease cholesterol production in human liver cells by inhibiting HMG-CoA reductase, specifically inhibit rumen methanogenic *Archaea*.

There are few reports on the effects of garlic extract on animal performance. Busquet et al. (2003) reported an increase of 1 L/animal/d in milk production compared to a control, but the difference was not significant. Yang et al. (2006) reported no effect of garlic powder on milk production and composition, although the effect may be different if the extract instead of the powder would have been used, because main active components identified are the result of transformation during oil extraction (Lawson, 1996).

### *3.5. Combinations of essential oils*

The additive, synergistic and (or) antagonistic effects of combined essential oils have been reported previously (Burt, 2004). Many commercial products on the market have combined one or more essential oils, but very limited research is available on the potential synergies among them. A blend of essential oils (CRINA®, dsm Nutritional Products, Heerlen, NL) containing thymol, eugenol, vanillin and limonene, among other compounds (patent EP 0646321 B1; Rossi, 1999) has been studied for its effects in vitro and in vivo. Several studies in situ (Newbold et al., 1999; Molero et al., 2004) observed that CRINA inhibited protein degradation, although changes reported were small and variable depending on the feed being degraded, the type of ration fed to the animals and the length of the adaptation period. Benchaar et al (2006b) reported no changes in in situ protein degradation of SBM samples in sheep or cattle supplemented with 0.11 or 2 g/d of CRINA. McIntosh et al. (2003) tested the proteolytic, peptidolytic and deaminase activity of ruminal fluid of dairy cows supplemented during 4 weeks with 1 g/d of CRINA and did not observe effects on proteolytic or peptidolytic activity, but ammonia production from casein degradation decreased, and researchers concluded that CRINA was likely inhibiting a specific group of gram-positive bacteria generally called hyper ammonia producing bacteria. This hypothesis was confirmed when McIntosh et al. (2003) observed that pure cultures of hyper ammonia producing bacteria, such as *Clostridium sticklandi* and *Peptstreptococcus anaerobius*, were very sensitive to the addition of CRINA. However, other ammonia-producing bacteria as *Prevotella ruminicola*, *Butyrivibrio fibrisolvens* or *Clostridium aminophilum* were less sensitive to CRINA. Wallace et al. (2002) also reported that ammonia production and hyper ammonia producing bacteria decreased in sheep fed this CRINA in a low protein diet. These effects were associated with a decrease in the microbial colonization of protein feeds. Most studies which have evaluated the effect of CRINA on rumen microbial fermentation have not observed effects on the synthesis of microbial protein (McIntosh et al., 2000; Wallace et al., 2002). Castillejos et al. (2005 and 2007) observed in vitro that CRINA increased total VFA production and acetate proportion. More recently, Benchaar et al. (2006b and 2007) reported that feeding this CRINA to dairy cattle increased ruminal pH and ADF digestion, but had no effects on VFA, N metabolism, protozoa counts or animal performance. Benchaar et al. (2006a) also reported no effects of the addition of CRINA on DM intake and growth rate of beef cattle.

The combination of cinnamaldehyde and eugenol (XTract Dairy, Pancosma, Geneve, Switzerland) has also been tested for its effects on rumen fermentation. Cardozo et al. (2006) reported that the combination of cinnamaldehyde (180 mg/d) plus eugenol (90 mg/d) reduced total DM and water intake in beef cattle. The reduction in DM intake was also observed in dairy cattle supplemented with high doses of cinnamaldehyde (500 mg/d; Busquet et al., 2003), and may be related to palatability problems, suggesting that the product needs to be encapsulated to overcome this problem. However, XTract Dairy treatment had no effects

on total and individual VFA concentrations, but increased the concentration of small peptide and amino acids, and decreased the concentration of ammonia-N, which suggests that XTract Dairy treatment inhibited deamination of AA. This reduction in deamination is consistent with results observed in vitro with clove bud, eugenol and cinnamaldehyde (Busquet et al., 2005ab; Cardozo et al., 2005). *Entodinium* spp. counts tended to decrease, and *Isotricha* spp. counts increased 3 h after feeding, suggesting that the cinnamaldehyde plus eugenol treatment had some effect on protozoa populations. It is likely that the limited effects of cinnamaldehyde and eugenol on rumen microbial fermentation were related to the relatively low dose used. In a second experiment using canulated beef cattle, Cardozo et al. (2006) fed XTract Dairy encapsulated to prevent effects on DM intake and at three times the dose fed in their previous study (600 mg/d of cinnamaldehyde and 300 mg of eugenol). Intake of DM was not affected, suggesting that encapsulation was successful in preventing palatability problems reported in previous studies. Furthermore, the effects of higher doses were more apparent; total VFA concentration was not affected by treatments, but the molar proportion of acetate decreased and the molar proportion of propionate increased. The XTract Dairy treatment resulted in the accumulation of small peptides and AA and the reduction of BCVFA and ammonia-N concentrations, suggesting that it inhibited deamination activity. The anti-protozoal effect of the cinnamaldehyde plus eugenol treatment was confirmed by the reduction in *Entodinium* spp. and *Isotricha* spp. counts 3 h after feeding, in agreement with the previous trial. These results are encouraging in the development of alternative products to ionophores for beef cattle, but other compounds or mixes may provide similar or better results.

## 4. Conclusions

Essential oils and their active components may have a strong antimicrobial activity against gram-positive and negative bacteria. At doses between 50 and 500 mg/L, some essential oils and their active components were able to modify rumen fermentation by changing VFA production and (or) protein metabolism. In general terms, the fermentation profile suggests that the main mechanism of action is the inhibition of methanogenesis and deamination. However, these effects may be diet and pH dependent. Based on the mechanisms of action of different additives on nutrient degradation and fermentation in the rumen, it is possible to identify potential synergies. A clear definition of the effects and target activities to modify, and a careful selection and combination of essential oils or their active components to modify these activities may provide a useful tool to improve the efficiency of nutrient utilization in the rumen. However, the limited scientific information published on the ruminal effects of many of the commonly used essential oils may result in confusion and inappropriate use of products and doses. Furthermore, there is an urgent need to conduct in vivo studies to determine the optimal dose and effects on animal performance.

## References


Benchaar, C., Duynisveld, J.L., Charmley, E., 2006a. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Can. J. Anim. Sci. 86, 91-96.

Benchaar, C., H. V. Petit, R. Berthiaume, T. D. Whyte, and P. Y. Chouinard. 2006b. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. J. Dairy Sci. 89:4352–4364.

Benchaar, C., S. Calsamilglia, G. R. Fraser, D. Colombatto, A. V. Chaves, K. A. Beauchemin, and T. A. McAllister. 2007. Use of plant-derived essential oils in ruminant nutrition: A review. Anim. Feed Sci. Technol. (In press).

Benchaar, C., H.V. Petit, R. Berthiaume, D.R. Ouellet, J. Chiquette, and P.Y. Chouinard. 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci. 90:886-897.

Borchers, R. 1965. Proteolytic activity of rumen fluid in vitro. J. Anim. Sci. 24:1033-1038.

Broderick, G. A., and J. E. Balthrop. 1979. Chemical inhibition of amino acid deamination by ruminal microbes in

vitro. J. Anim. Sci. 49:1101-1111.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food. Microbiol. 94:223-253.

Busquet, M., H. Greathead, S. Calsamiglia, A. Ferret, and C. Kamel. 2003. Efecto del extracto de ajo y el cinemaldehido sobre la producción, composición y residuos en leche en vacas de alta producción. ITEA, 24 (Vol. Extra):756-758.

Busquet, M., S. Calsamiglia, A. Ferret, and C. Kamel. 2005a. Screening for the effects of natural plant extracts and secondary plant metabolites on rumen microbial fermentation in continuous culture. Anim. Feed Sci. Technol. 123/124:597-613.

Busquet, M., S. Calsamiglia, A. Ferret, P. W. Cardozo, and C. Kamel. 2005b. Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. J. Dairy Sci. 88:2508-2516.

Busquet, M., S. Calsamiglia, A. Ferret, M. D. Carro, and C. Kamel. 2005c. Effect of garlic oil and four of its compounds on rumen microbial fermentation. J. Dairy Sci. 88:4393-4404.

Busquet, M., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Plant extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89:761-771.

Calixto J. B., A. Beirith, J. Ferreira, A. R. S. Santos, V. C. Filho, and R. A. Yunes. 2000. Naturally occurring antinociceptive substances from plants: a review. Phytother. Res. 14:401-418.

Calsamiglia, S., M. Busquet, P. W. Cardozo, L. Castillejos, and A. Ferret. 2007. Essential oils for modifying rumen fermentation: A review. J. Dairy Sci. 90:2580-2595.

Calsamiglia, S., L. Castillejos, and M. Busquet. 2006. Alternatives to antimicrobial growth promoters in cattle. Pages 129-167 in Recent Advances in Animal Nutrition. P.C. Garnsworthy, and J. Wiseman, ed. Nottingham University Press, Nottingham, UK.

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2004. Effects of natural plant extracts on protein degradation and fermentation profiles in continuous culture. J. Anim. Sci. 82:3230-3236.

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2005. Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle . J. Anim. Sci. 83:2572-2579.

Cardozo, P. W., S. Calsamiglia, A. Ferret, and C. Kamel. 2006. Effects of alfalfa extract, anise, capsicum and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high concentrate diet. J. Anim. Sci. 84:2801-2808.

Castillejos, L., S. Calsamiglia, A. Ferret, and R. Losa. 2005. Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Anim. Feed Sci. Technol. 119:29-41.

Castillejos, L., S. Calsamiglia, and A. Ferret. 2006. Effect of essential oils active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. J. Dairy. Sci. 89:2649-2658.

Castillejos, L., S. Calsamiglia, A. Ferret, and R. Losa. 2007. Effects of dose and adaptation time of a specific blend of essential oils compounds on rumen fermentation. Anim. Feed Sci. Technol. 132:186-201.

Chalupa, W., W. Corbett, and J. R. Brethour. 1980. Effects of monensin and amicloral on rumen fermentation. J. Anim. Sci. 51:170-179.

Chao, S. C., and D. G. Young. 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J. Essent. Oil Res. 12:639-649.

Cichewicz, R. H., and P. A. Thorpe.1996. The antimicrobial properties of chile peppers (*Capsicum* species) and their uses in Mayan medicine. J. Ethnopharmacol. 52:1-70.

Cimanga, K., K. Kambu, L. Tona, S. Apers, T. Bruyne, N. Hermans, J. Totté, L. Pieters, and A. J. Vlietinck. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Replublic of Congo. J. Ethnopharmacol. 79:213-220.

Cox, S. D., C. M. Mann, and J. L. Markam. 2001. Interaction between components of the essential oil of *Melaleuca alternifolia*. J. Appl. Microbiol. 91:492-497.

Davidson, P. M., and A. S. Naidu. 2000. Phyto-phenols. Pages 265-293 in Natural Food Antimicrobial Systems. A. S. Naidu, ed. CRC Press, Boca Raton, FL.

Deans, S. G., and G. Ritchie. 1987. Antibacterial properties of plant essential oils. Int. J. Food Microbiol. 5:165-180.

Dorman, H. J. D., and S. G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88:308-316.

Evans, J. D., and S. A. Martin. 2000. Effects of thymol on ruminal microorganisms. Curr. Microbiol. 41:336-340.

Fandiño, I., S. Calsamiglia, A. Ferret, C. Kamel. 2006. Anise and capsicum as alternative to monensin in beef heifers fed a high-concentrate diet. J. Dairy Sci. 89(Suppl. 1):T237.

Fandiño, I., S. Calsamiglia, A. Ferret, C. Kamel. 2006. Optimal dose and combination of anise and capsicum as modifiers of ruminal fermentation in beef heifers. J. Dairy Sci. 89(Suppl. 1) :T238.

Feldberg, R. S., S. C. Chang, A. N. Kotik, M. Nadler, Z. Neuwirth, D. C. Sundstrom, and N. H. Thompson. 1988. In vitro mechanism of inhibition of bacterial cell growth by allicin. Antim. Agents Chem. 32:1763-1768.

Ferme, D., M. Banjac, S. Calsamiglia, M. Busquet , C. Kamel, and G. Avgustin. 2004. The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. Folia Microbiol., 49:151-5.

FDA, 2005. Freedom of information summary. Supplemental new animal drug application. NADA 095-735. Monensin Sodium (RUMENSIN 80): Type A Medicated Article for Dairy Cattle. http://www.fda.gov/cvm/FOI/095-735s121505.pdf

Gebhart, R., and H. Beck. 1996. Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. Lipids. 31:1269-1276.

Gershenzon, J., and R. Croteau. 1991. Terpenoids. Pages 165-219 in Herbivores: their interactions with secondary plant metabolites, Vol. 1. G. A. Rosenthal, and M. R. Berenbaum, ed. Academic Press, San Diego, CA.

Griffin, S. G., S. G. Wyllie, J. L. Markham, and D. N. Leach. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flavour Fragr. J. 14:322-332.

Gustafson, R. H., and R. E. Bowen. 1997. Antibiotic use in animal agriculture. J. Appl. Microbiol. 83: 531-541.

Gutiérrez, M. E., A. F. García, M. A. de Madariaga, M. L. Sagrista, F. J. Casadó, and M. Mora. 2003. Interaction of tocopherols and phenolic compounds with membrane lipid components: evaluation of their antioxidant activity in a liposomal model system. Life Sciences. 72:2337-2360.

Hammer, K. A., C. F. Carson, and T. V. Riley. 1999. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985-990.

Harborne, J. B. and C. A. Williams. 2000. Advances in flavonoid research since 1992. Phytochem. 55:481-504.

Helander, I. M., H. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E. J. Smid, L. G. M. Gorris, and A. Wright. 1998. Characteritzation of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food Chem. 46:3590-3595.

Hino, T., and J. B. Russell. 1985. Effect of reducing-equivalent disposal and NADH/NAD on deamination of amino acids by intact rumen microorganisms and their cell extracts. Appl. Environ. Microbiol. 50:1368-1374.

Hoffmann, C., and C. A. Evans. 1911. The use of spices as preservatives. J. Ind. Eng. Chem. 3:835-838.

Horton, G. M. J. 1980. Use of feed additives to reduce ruminal methane production and deaminase activity in steers. J. Anim. Sci. 50:1160-1164.

Juven, B. J., J. Kanner, F. Schved, and H. Weisslowicz. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J. Appl. Bacter. 76:626-631.

Kamel, C., H.M.R. Greathead, M.L. Tejido, M.J. Ranilla and M.D. Carro. 2007. Effects of allicin and diallyl disulfide on in vitro rumen fermentation of a mixed diet. Anim. Feed Sci. Technol. (In Press).

Kamel, C. 2001. Tracing modes of action and the roles of plant extracts in non-ruminants. Pages 135-150 in Recent advances in animal nutrition. P. C. Garnsworthy, and J. Wiseman, ed. Nothingham University Press, Nothingam, UK.

Lawson, L. 1996. The composition and chemistry of garlic cloves and processed garlic. Pages 37-107 in Garlic. The Science and Therapeutic Application of *Allium sativum* L. and Related Spieces. H. P. Koch, and L. D. Lawson, ed. Williams & Wilkins, Baltimore, MD.

Lee, K. G., and T. Shibamoto. 2001. Antioxidant property of aroma extract isolated from clove buds [*Syzgium aromaticum* (L.) Merr. et Perry]. Food Chem. 74:443-448.

Lee, S. E., H. J. Hwang, J. S. Ha, H. S. Jeong, and J. H. Kim. 2003. Screening of medicinal plant extracts for antioxidant activity. Life Sci. 73:167-179.

Litchenthanler, H. K. 1999. DOXP-pathway of plant isoprenoid bioshynthesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 50:45-63.

Mahmoud, A. L. E. 1994. Antifungal action and antiaflatoxigenic properties of some essential oil constituents. Let. Appl. Microbiol. 19:110-113.

Mansfield, H. R., M. I. Endres and M. D. Stern. 1995. Comparison of microbial fermentation in the rumen of dairy cows and dual flow continuous culture. Anim. Feed Sci. Technol. 55:47-66.

Marino, M., C. Bersani, G. Comi. 2001. Impedance measurements to study the antimicrobial activity of essential oils from Lamiacea and Compositae. Int. J. Food Microbiol. 67, 187– 195.

Martin, S. A., and J. M. Macy. 1985. Effects of monensin, pyromellitic diimide and 2-bromoethanesulfonic acid on rumen fermentation in vitro. J. Anim. Sci. 60:544-550.

McIntosh, F. M., C. J. Newbold, R. Losa, P. Williams, and R. J. Wallace. 2000. Effects of essential oils on rumen fermentation. Reprod. Nutr. Dev. 40:221-222.

McIntosh, F. M., P. Williams, R. Losa, R. J. Wallace, D. A. Beever, and C. J. Newbold. 2003. Effects of essential oils on ruminal microorganisms and their protein metabolism. Appl. Environ. Microbiol. 69:5011-5014.

Miller, T. L., and M. J. Wollin. 2001. Inhibition of growth of

methane-producing bacteria of the ruminant forestomach by Hydroxymethylglutaryl-SCoA reductase inhibitors. J. Dairy Sci. 84:1445-1448.

Molero, R., M. Ibars, S. Calsamiglia, A. Ferret, and R. Losa. 2004. Effect of a specific blend of essential oil compounds on dry matter and crude protein degradability in heifers fed diets with different forage to concentrate rations. Anim. Feed Sci. Technol. 114:91-104.

Nagy, J. G., and R. P. Tengerdy. 1968. Antibacterial action of essential oils of *Artemisia* as an ecological factor. II. Antibacterial action of the volatile oils of *Artemisia tridentata* (Big Sagebrush) on bacteria from the rumen of mule deer. Appl. Microbiol. 16:441-444.

Newbold, C. J., F. M. McIntosch, P. Williams, R. Losa, and R. J. Wallace. 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol. 114:105-112.

Oh, H. K., T. Sakai, M. B. Jones, and W. M. Longhurst. 1967. Effect of various essential oils isolated from Douglas Fir Needles upon sheep and deer rumen microbial activity. Appl. Microbiol. 15:777-784.

Oh, H. K., M. B. Jones, and W. M. Longhurst. 1968. Comparison of rumen microbial inhibition resulting from various essential oils isolated from relatively unpalatable plant species. Appl. Microbiol. 16:39-44.

Pentz, R., and C. P. Siegers. 1996. Methods for qualitative and quantitative assessment of their ingredients. Pages 109-134 in Garlic. The Science and Therapeutic Application of *Allium sativum* L. and Related Species. H. P. Koch, and L. D. Lawson, ed. Williams & Wilkins, Baltimore, MD.

Reddy, L., B. Odhav, and K. D. Bhoola. 2003. Natural products for cancer prevention: a global perspective. Pharmacol. Therap. 99:1-13.

Reuter, H. D., J. P. Koch, and L. Lawson. 1996. Therapeutic effects and applications of garlic and its preparations. Pages 135-212 in Garlic. The Science and Therapeutic Application of *Allium sativum* L. and Related Species. H. P. Koch, and L. D. Lawson. ed. Williams & Wilkins, Baltimore, MD.

Ross, Z. M., E. A. O'Gara, D. J. Hill, H. V. Sleightholme, and D. J. Maslin. 2001. Antimicrobial properties of garlic oil against human enteric bacteria: evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbiol. 67:475-480.

Rossi, J. 1999. Additives for animal nutrition and technique for their preparation. European patent EP 0646321 B1.

Sangwan, N. S., A. H. A. Farooqi, F. Shabih, and R. S. Sangwan. 2001. Regulation of essential oil production in plants. Plant Growth Reg. 34:3-21.

Sivropoulou, A., E. Papanikolau, C. Nikolau, S. Kokkini, T. Lanaras, and M. Arsenakis. 1996. Antimicrobial and cytotoxic activities of *Origanum* Essential Oils. J. Agric. Food Chem. 44:1202-1205.

Skandamis, P. N., and G. J. Nychas. 2000. Development and evaluation of a model predicting the survival of *Escherichia coli* O157:H17 NCTC 12900 in homemade eggplant salad at various temperatures, pHs, and oregano essential oil concentration. Appl. Envirom. Microbiol. 66:1646-1653.

Smith-Palmer, A., J. Stewart, and L. Fyfe. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Let. Appl. Microbiol. 26:118-122.

Tamminga, S. 1996. A review on environmental impacts of nutritional strategies in ruminants. J. Anim. Sci. 74: 3112-3124.

Trouillas, P., C. A. Calliste, D. P. Allais, A. Simon, A. Marfak, C. Delage, and J. L. Duroux. 2003. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. Food Chem. 80:399-407.

Ultee, A., E.P. Kets, and E. J. Smid. 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 65:4606-4610.

Ultee, A., M. H. J. Bennik, and R. Moezelaar. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 68:1561-1568.

Van Nevel, C. J., and D. I. Demeyer. 1988. Manipulation of rumen fermentation. Pages 387-443 in The Rumen Microbial Ecosystem. P. N. Hobson, ed. Elsevier Applied Science, NY.

Wallace, R. J., N. R. McEwan, F. M. McIntosh, B. Teferedegne, and C. J. Newbold. 2002. Natural products as manipulators of rumen fermentation. Asian-Austr. J. Anim. Sci. 10:1458-1468.

Yang, W.Z., Chaves, A.V., He, M.L., Benchaar, C., McAllister, T.A., 2006. Effect of monensin and essential oil on feed intake, milk yield and composition of lactating dairy cows. Can. J. Anim. Sci. 86, 598 (Abstr).

Zafra, M. A., F. Molina, and A. Puerto. 2003. Effects of perivagal administration of capsaicin on post-surgical food intake. Auto. Neurosci.: Basic and Clin. 107:37-44.

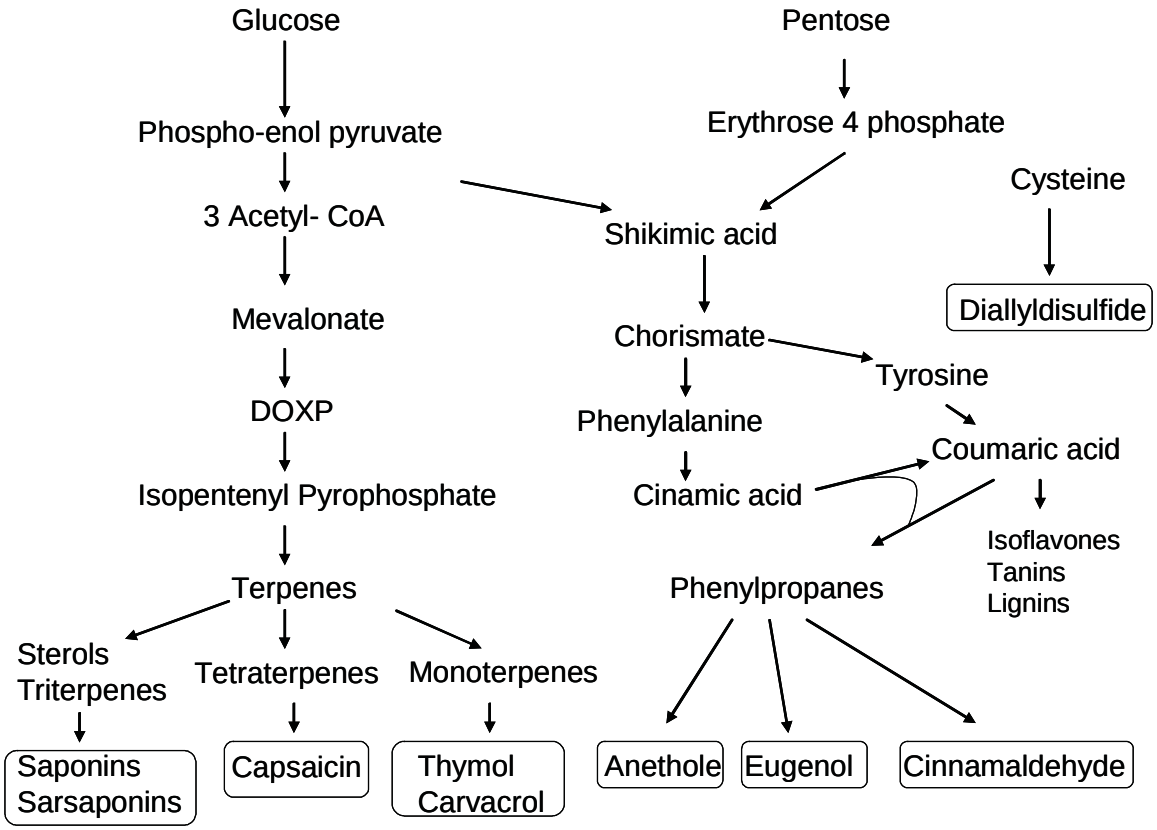


**Figure 1.** Metabolic pathways of the biosynthesis of main plant extract active components (adapted from Calsamiglia et al., 2007).

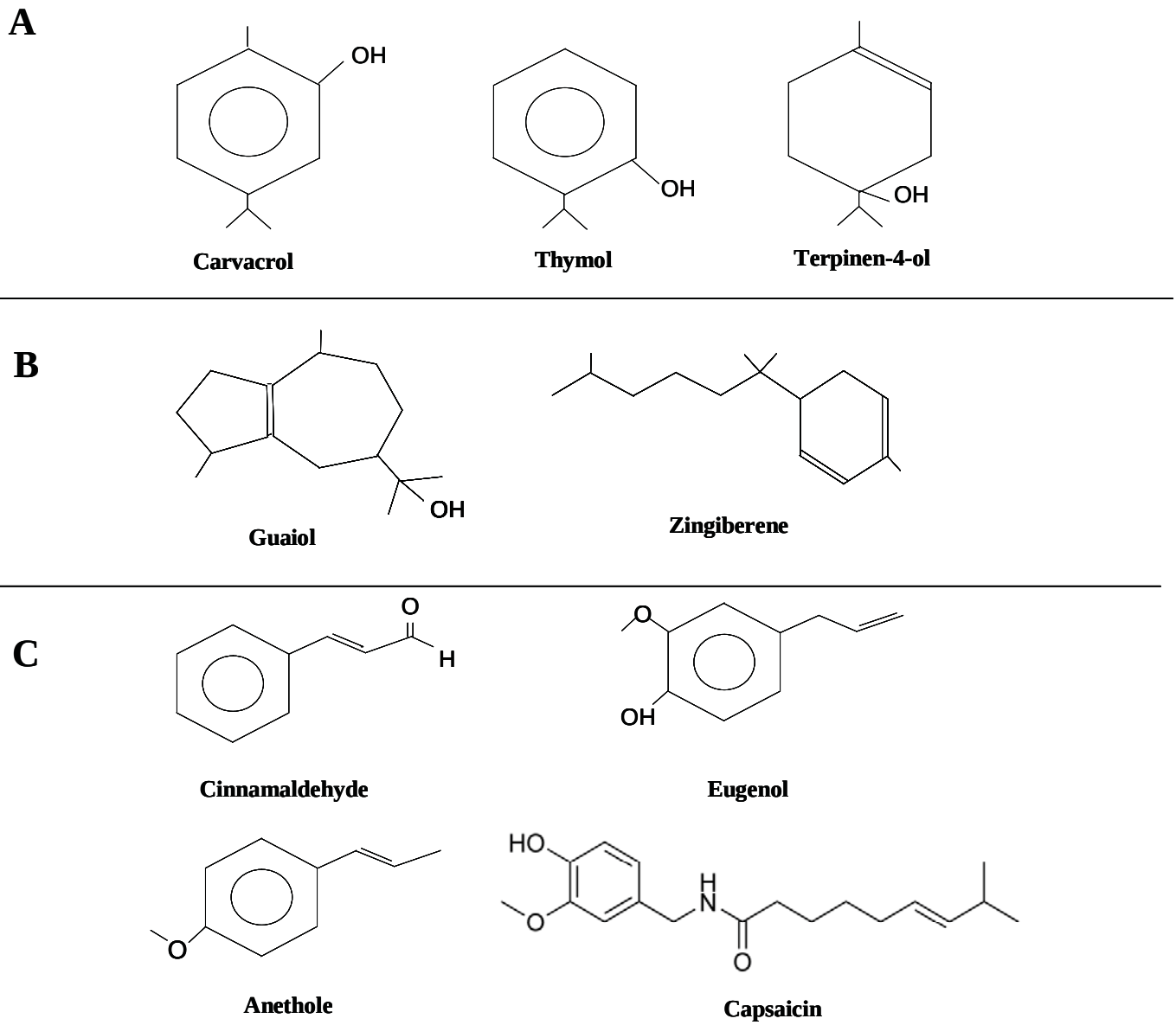


**Figure 2.** Main monoterpenoid (A), sesquiterpenoids (B) and phenylpropanoid (C) components of essential oils (adapted from Calsamiglia et al., 2007).

**Table 1.** Essential oils with antimicrobial activity, their main active components and susceptible microorganisms (adapted from Calsamiglia et al, 2007)

| Essential oil of | Name | Active components | Susceptible microorganisms | Reference |
|---|---|---|---|---|
| *Allium sativum* | Garlic | Allicin, Diallyl sulfite | Enteropatogenic bacteria | Ross et al., 2001 |
| *Anethum graveolens* | Dill | Limonene, Carvone | Gram-positive and Gram-negative bacteria | Deans and Ritchie, 1987 |
| *Capsicum annum* | Paprika | Capsaicin | Gram-positive and Gram-negative bacteria | Deans and Ritchie, 1987 |
| *Cinnamomum cassia* | Cassia | Cinnamaldehyde | *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis* | Smith-Palmer et al., 1998; Mahmoud, 1994; Ouattara et al., 1997 |
| *Juniperus oxycedrus* | Juniper | Cadinene, Pinene | *Aeromonas sobria, Enterococcus fecalis, Staphylococcus aureus* | Hammer et al.,1999 |
| *Melaleuca alternifolia* | Tea tree | Terpinen-4-ol | *S. aureus, E. col,* Gram-positive and Gram-negative bacteria | Cox et al.,2001; Chao and Young, 2000 |
| *Origanum vulgare* | Oregano | Carvacrol, Thymol | Gram-positive and Gram-negative bacteria | Dorman and Deans, 2000; Sivropoulou et al., 1996 |
| *Pinpinella anisum* | Anise | Anethol | *Aeromonas hydrophila, Brevibacterium linens, Brochothrics thermosphacta* | Deans and Ritchie, 1987 |
| *Rosmarinus officinalis* | Rosemary | 1,8-Cineole | *Staphylococcus aureus, Listeria monocytogens, Campylobacter jejuni* | Smith-Palmer et al., 1998; Ouattara et al., 1997 |
| *Syzygium aromaticum* | Clove | Eugenol | *E. coli, S. aureus, L. monocytogenes, S. enteritidis, C. jejuni* | Smith-Palmer et al., 1998; Ouattara et al., 1997 |
| *Thymus vulgaris* | Thyme | Thymol, Carvacrol | *Salmonella thypimurium, S. aureus, A. flavus* | Ouattara et al., 1997; Juven et al., 1994; Mahmoud, 1994 |
| *Zingiber officinale* | Ginger | Zingiberene, Zingerone | Gram-positive and Gram-negative bacteria | Chao and Young, 2000 |