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Original research paper

ESSENTIAL OIL QUALITY INFLUENCED BY FEED PELLETING PROCESSING

Ljiljana Kostadinović[1,*], Sanja Popović[2], Ivana Čabarkapa[2], Jovanka Lević[2]

[1]Planet Fresh, Podgorički put bb, 81402 Nikšić, Montenegro
[2]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*Corresponding author:
E-mail address: ljiljana.m.medjedovic@gmail.com; info@planet-fresh.com

ABSTRACT: From antiquity, essential oils of medical plants and their derivatives have being used for flavoring foods and beverages and for medication. These additives have been usefully used in animal nutrition for the improvement of health and animal wellbeing since they have high antimicrobial and antioxidant activities. In the present study, the effect of the pelleting process on essential oil composition and stability was investigated, since they are very unstable during thermal processing which is widely used these days in feedstuff production. The composition of essential oil obtained by hydrodistillation from the plant *Origanum vulgare*, which was added into feed for broilers in the concentration of 2 g/kg, was analyzed by GC/FID before and after the pelleting process. After the pelleting of feed, the essential oil was also isolated from the animal diet by hydrodistillation. Analysis of essential oils obtained before and after the pelleting process showed some quantitative differences. Oregano essential oil was characterized by the presence of thymol (19.9%) and carvacrol (61.8%) at the beginning of the experiment. After the pelleting process, the concentration of thymol and carvacrol amounted to 15.3% and 50.4%, respectively. It was concluded that the pelleting process had a significant effect on thymol and carvacrol stability in animal feed, i.e. on reducing their initial contents in animal feed.

Key words: *feed, pelleting, essential oil, stability*

INTRODUCTION

Parts of plants, or the products thereof have been used since ancient times in the diet of humans and animals in order to improve the flavor of food, as well as in medical purposes, for the prevention or treatment of various diseases (Burt, 2004; Negi, 2012; Puvača et al., 2018). In recent years, numerous studies were dealing with the isolation of bioactive compounds from plants, which are known to have antioxidant, antimicrobial, or some other beneficial effects (Burt, 2004). Whether it comes to herbal extracts, essential oils, or organic acids, these natural products are a good alternative to artificial preparations, finding more and more use in the production of animal feed, primarily in order to increase food safety. Carvacrol (5-isopropyl-2-methylphenol) is one of the main components of oregano oil with proven antioxidant, antifungal and insecticidal effect (Burt, 2004; Chami et al., 2005; Panella et al., 2005; Tampieri et al., 2005; Liolios et al., 2009). In addition to antioxidants, carvacrol has well known antimicrobial activity. The exact mechanism of action of this compound on microorganisms is not yet known, but the majority of scientific studies indicate that its activity is based on the destruction of the cell membranes of microorganisms (Helander et al., 1998; Inamuco, 2012; Aćimović et al., 2019). Thymol (5-methyl-2-isopropylphenol) is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides such as carvacrol. Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties (Zarrini et al., 2010). Compounds in the essential oils of one type of oregano have demonstrated antimutagenic effects, and in particular carvacrol and thymol demonstrated a strong antimutagenic effect (Mezzoug et al., 2007). In the past decade, the use of aromatic herbs, essential oils and plant extracts became a special interest in animal nutrition, initially because it was established that these drugs can effectively replace antibiotics and growth promoters and for their the other positive effects (Vieira et al., 2008; Kostadinović and Lević, 2018). On the other side, the processing of feedstuffs before ingestion, to improve digestibility, has become a major focus in nutrition researches. Animal feed manufacturing involves the use of a variety of feed technological processes like pelleting, expanding and extrusion to produce compound feeds (Riaz, 2000). Nearly every one of these operations can have a positive influence on essential ingredients in feed, which is their primary aim but can also have adverse effects. Hereby, the process should be optimized so that products meet the required nutritional quality of animal feed. Essential oils are sensitive materials that can easily suffer degradation under the action of oxygen, light and moderate temperatures (Puvača et al., 2019). Therefore, an adequate application of the essential oil which takes into account these aspects is required for commercial use. Because of all above mentioned, this study was carried out to determine if the pelleting process affects the stability of thymol and carvacrol present in essential oil isolated from *Origanum vulgare*, which was added into the feed for broilers.

MATERIAL AND METHODS

The essential oil was isolated from dried plant material by hydrodistillation, according to the standard procedure reported in the Sixth European Pharmacopoeia (2008). Along with the Clevenger type apparatus, the duration of distillation was 2 hours. Oil samples were dried with anhydrous sodium sulfate, dissolved in ethanol and analyzed by GC/FID.

The essential oil samples were analyzed by a gas chromatograph Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector (GC-FID), auto-injection module for liquid, equipped with fused silica capillary column (DB–WAX 30 m, 0.25 mm, 0.50 μm). The carrier gas was helium (purity>99.9997 vol%, produced by Messer, Germany), at a flow rate of 1.26 mL/min. The composition of essential oil was determined by comparing the retention times of oil components (thymol or carvacrol) with those of standards from the data library. Results were expressed as the mass of thymol or carvacrol (g) per 100 g of essential oil.

The animal feed used in this study was a starter diet mixture for broilers, whose composition is shown in Table 1.

In starter, the diet mixture was added 2 g/kg of essential oil obtained from the plant *Origanum vulgare*.

The starter diet mixture for broilers was conditioned in double-shaft steam conditioner Muyang SLHSJ0.2A (China) until material reached temperature of 80°C. Water was directly added to feed mash during conditioning. After the conditioning process, the moisture content of feed for broilers was 15.8%.

Complete mixture for broilers was pelleted in a flat die pellet press 14-175, AMANDUS KAHL GmbH & Co. KG (Germany). A die with a 6 mm diameter of the openings and with a pressing way of 36 mm was used (diameter to length ratio 1:6). The pellets were collected at a pelleting temperature of 60°C. The speed of passage of material was 18.6 kg/h.

After pelleting, the product was stored for 24 hours under room conditions in order to achieve stable temperature and subsequently milled by hummer mill with a sieve opening of 4 mm.

Results are presented in the tables as the mean ± standard deviations (SD) of a number (n) of independent determinations. The one way ANOVA analysis (STATISTICA 12.0) was performed to assess data differences among various groups.

Table 1. Composition of starter diet mixture for broilers

Ingredient	**Share, %**
Corn flour	41.8
Soybean meal	37.2
Full fat soya	12.5
Soybean oil	4.0
Monocalcium phosphate	1.4
Dl Methionine	0.3
Limestone	1.6
Lysine	0.2
Premix*	1.0

*Vitamins and minerals provided per kilogram of diet: Vitamin A, 3600000 IU; Vitamin B1, 720 mg; Vitamin B2, 2640 mg; Pantothenic acid, 4000 mg; Nicotinic acid, 12000 mg; Vitamin B6, 1200 mg; Folic acid, 400 mg; Vitamin B12, 6 mg; Vitamin D3, 800000; Vitamin E, 7200 IU; Vitamin K3, 800 mg; Biotin, 40 mg; Antioxidant, 100000 mg; Choline chloride, 5000 mg; Manganese, 40000 mg; Zinc, 33880 mg; Iron, 20000 mg; Copper, 4000 mg; Iodine, 400 mg; Selenium, 80 mg.

RESULTS AND DISCUSSION

The composition of essential oil from *Origanum vulgare* was characterized by the dominant presence of two substances, thymol, and carvacrol. The essential oil composition before the pelleting process and after thermal processing is shown in Table 2.

Table 2. The essential oil composition before and after the pelleting process

Treatment	**Essential oil compositions**			
	γ-terpinene	p-cymene	carvacrol	thymol
Before pelleting	3.1 ± 0.2^{a}	11.6 ± 0.4^{a}	61.8 ± 1.1^{b}	19.9 ± 0.7^{b}
After pelleting	2.9 ± 0.3^{a}	10.7 ± 0.2^{b}	50.4 ± 0.9^{a}	15.3 ± 0.2^{a}

Values are presented as means ±SD of six replicates; $^{a-b}$ Means within a column with no common superscript differ significantly at $p<0.05$.

Most of the processes used in feed manufacturing are designed to increase the value of feed ingredients, to improve growth rate, the efficiency of gain, nutrient digestibility, and to ameliorate feed manipulation (Fairfield, 2003). On the other hand, these processes are harmful to labile nutrients that can be easily damaged (Dozier, 2002), which was proved by the results obtained in this study. In order to establish a more suitable and payable way of essential oil application, it was necessary to investigate to what extent this thermal process affects the essential oil composition. At the beginning of the study, the initial concentration of thymol incomplete mixture for broilers was around 19.9 %. In contrast to this, the concentration of thymol in pelleted feed for broilers decreased by 76.9% of its initial value. The initial concentration of carvacrol in essential oil obtained from the *Origanum vulgare* plant was 61.8%. Following the process of pelleting a statistically significant ($p<0.05$) reduction of carvacrol content in the feed of broilers was observed. The concentration of carvacrol after thermal processing amounted to 50.4%, which shows that the reduction of carvacrol concentration after pelleting was nearly 18.5% of its initial value. Comparing the percentage of reduction in the content of thymol and carvacrol, it has been found that thymol is slightly more sensitive than carvacrol, in terms of high temperature, humidity, and pressure. These results show that the pelleting process has a greater impact on the degradation of thymol compared to carvacrol. Nowadays, there is a wide range of macro and micro components in liquid form and increasing trend in the feed industry to use them. In order to achieve a high quality of feed, and at the same time as animal products, development of suitable technical equipment for their application is absolutely necessary. New technologies are developed to achieve: better pellet quality, higher liquid contents in pellets, better protection of heat-sensitive micro-ingredients. Vacuum-coating is a possible technological tool for the post-pelleting application of liquids in animal feed. On the other side, common goals in the development of essential oil formulations are to protect the essential oil from degradation or from losses by evaporation, to achieve a controlled release, and to facilitate handling. This problem can be overcome by producing a dry formulation by microencapsulation. This is a relatively new technology intended for protection, stabilization, and slow dismissal of food ingredients. The encapsulating is used generally for starch, proteins, gums, lipids, essential oils or any combination of them. Methods of encapsulation of food ingredients include spray-drying, freeze-drying, co-crystallization, and molecular inclusion.

Therefore, all the above-mentioned types of liquid applications may be a possible solution for essential oil applications in animal feed, in order to achieve high-quality feed, which has a positive effect on animal health, and hence on the people's health.

CONCLUSION

Based on the obtained results it can be concluded that the pelleting process had an impact on the degradation on examined components of essential oil, thymol, and carvacrol and that losses of mentioned components in treated feeds were generally high. The average reduction of thymol and carvacrol was about 23.1% and around 18.5%, respectively. This is an important difference in terms of nutritional, but economic aspects also. A possible solution to this problem may be the addition of essential oil in higher concentrations, in which case the contents of examined components would decrease to the desired value after the technological processing of animal feed. Furthermore, future studies should consider some other possible ways of essential oil application into animal feed, which will not have a negative impact on thymol and carvacrol stability.

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