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Effect of adding essential oils of coriander (*Coriandrum sativum* L.) and hyssop (*Hyssopus officinalis* L.) on the shelf life of ground beef

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ABSTRACT

This study examined the effect of adding essential oils of hyssop and coriander at the highest concentration (0.02% v/w) sensorially acceptable to a panel of assessors on the microbiological and biochemical characteristics of stored ground beef. Vacuum-packed meat was stored at 0.5 ± 0.5 °C and 6 ± 1 °C for 15 days. The greatest beneficial effect of both additives was in inhibiting the development of undesirable sensory changes (extending acceptability by up to 3 days) and the growth of *Enterobacteriaceae* (by up to approximately 1–2 log cycles compared with the controls). The effect on lactic acid bacteria, total viable bacterial count and other groups of microorganisms investigated was minor (up to 1 log cycle) and similar for both oils. Neither did these additives significantly affect amino nitrogen levels, protease activity, the proportions of meat pigments, protein electropherograms and pH levels. This indicates the limited effect of these essential oils in the concentrations applied on preserving vacuum-packed minced beef.

1. Introduction

Minced beef is highly perishable. Indications of stored meat spoilage include microbiota activity, changes resulting from the activity of endogenous enzymes, and oxidative reactions, for example, of pigments and lipids. The shelf life of minced meat can be extended by the use of preservatives and other antimicrobial treatments (Fik & Leszczyńska-Fik, 2007; Fik, Surówka, & Firek, 2008). Essential oils, as components of herbs and spices, form a very large group of substances known for their antimicrobial properties. Their use as preservatives is not controversial since they are plant-based and have long been used in the kitchen as well as in herbal medicine. However, the application of such oils on an industrial scale in food preservation is not a clear-cut issue. Their usability is determined by two factors: how effective they are in terms of the product; and whether such modified products are acceptable to consumers. Consumer acceptance depends, in turn, both on the sensory qualities of the product and the absence of any tendency to induce allergic or sensitive reactions in the vast majority of consumers. Of about 3000 essential oils known at present, approximately 300 show commercial potential (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). Most of the papers studying the effect of essential oils on the inhibition of bacterial growth have investigated either strains isolated on culture media or the growth of selected strains, mainly pathogens, inoculated into a food product. These include *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Shigella* spp. (Bagamboula, Uyttendaele, & Debevere, 2004; Burt, 2004; Cvijovic, Djukic, Mandic, Acamovic-Djokovic, & Pesakovic, 2010; Gill, Delaquis, Russo, & Holley, 2002; Holley & Patel, 2005; Skandamis & Nychas, 2000; Skandamis, Tsigaria & Nychas, 2000; Skandamis, Tsigarida & Nychas, 2002). A relatively small number of papers refer to the effect of essential oils on the stability of a product and its native microbiota. In a complex biological system such as food various interactions may occur between the additives used, the constituents of a food and its matrix. This, in turn, may affect the effectiveness of essential oils with respect to food microorganisms (Burt, 2004; Gutierrez, Barry-Ryan, & Bourke, 2009; Skandamis et al., 2000). In meat and fish products, a high fat content, among other factors, has the effect of impairing the efficacy of essential oils (Burt, 2004). For example, Skandamis et al. (2000) found that the inhibition of *Salmonella typhimurium* with essential oil of oregano (0.03%) was far stronger on a liquid than on a solid medium. The effectiveness of alternative plant-derived preservatives may also depend on the temperature of product storage (Govaris, Solomakos, Pexara, & Chatzopoulou, 2010; Szczawińska, Czapska, Szczawiński, & Jackowska-Tracz, 2008).

Moreover, usually food is simultaneously colonized by many different strains of bacteria. Their interactions have an effect not only on the nature of spoilage, but also on whether the growth of individual strains is stimulated or inhibited. Such interactions between bacterial strains can be mutually antagonistic or can result in bacteria providing nutrients for each other and creating conditions for growth or using the capability of Gram-negative bacteria to coordinate the expression of certain phenotypic traits through bacterial communication (Gram et al., 2002).

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In general, higher concentrations of spice are required to inhibit microbial growth in food than in culture media (Shelef, 1984). One problem frequently encountered concerning the application of such additives in food is that the amount of essential oils' compounds necessary for effective food preservation exceeds the sensorially acceptable level (Nazer, Kobilinsky, Tholozan, & Dubois-Brissonnet, 2005). In previous studies on the use of essential oils to inhibit bacterial growth relatively high doses of such substances were generally recommended (Skandamis & Nychas, 2001; Skandamis et al., 2002; Solomakos, Govaris, Koidis, & Botsoglou, 2008).

The aim of this paper was to investigate whether and to what extent essential oils of coriander and hyssop, applied at the highest sensorially acceptable level, are able to extend the shelf life of vacuum-packed minced beef. The effect of these additives was analysed using multivariate analysis of variance (MANOVA), which, as well as other related methods, is widely used in analysing food products and how they are affected by specific factors (Arvanitoyannis & Van Houwelingen-Koukaliaroglou, 2003; Papadima, Arvanitoyannis, Bloukas, & Fournitzis, 1999; Tzouros & Arvanitoyannis, 2001).

2. Materials and methods

2.1. Materials

The experimental material was beef meat (muscles: semitendinous, biceps, class 1). Meat was collected directly from a slaughter house 1 day after slaughtering. Next, beef was minced using a MADO Primus MEW 613 meat grinder (Ø 4 mm). After grinding, the material was divided into three batches. One was left as the control batch (without oils). Of the remaining two batches, one was mixed with essential oil of hyssop (0.02% v/w) and the other with oil of coriander (0.02% v/w). The oils were subjected to GC/MS analysis to investigate their composition. The concentration of oils applied was established on the basis of results obtained in an earlier sensory evaluation and were the highest values accepted by a sensory panel augmented by 21 untrained volunteers. Next, the samples, divided into 0.4 kg portions, were vacuum packed in high-barrier Opalen HB 65 bags using a Vac-Star 1000 packaging machine. Half of the samples were stored at 6 ± 1 °C and the remainder at 0.5 ± 0.5 °C, in both cases without exposure to light. Analyses were carried out on the fresh raw material and then every three days during the 15-day storage period.

2.2. Methods

2.2.1. Analysis of essential oils

The 100% natural essential oils were purchased from a shop. Composition of essential oils was determined by a Varian 450-GC gas chromatograph, equipped with a Varian 320 MS mass detector. Separations were carried out on a VF-5ms column with a carrier gas flow rate of 0.5 ml/min, split mode 1:100 and injector temperature of 250 °C. The following heating programme was applied: 50 °C for 1 min; an increase of temperature up to 250 °C at a rate of 4 °C/min; and maintaining this temperature for 10 min. The range of mass analysed by a detector was 40–400 m/z at a scan speed of 0.8 s/scan. Kovats retention indexes were calculated on the basis of alcane series (C10–C40) (Van Den Dool & Kratz, 1963).

2.2.2. Sensory evaluation

The sensory evaluation included the odour of raw meat as well as the odour and taste of cooked meat, which was cooked in water for 15 min in the form of 50 g meatballs. Evaluation was performed by a 7-member sensory panel, trained and proven in sensory sensitivity (PN-ISO 8586-1, 1996). Samples served in random sequence on stoneware plates at a temperature of about 45 °C were each evaluated three times in the laboratory. White bread and water were provided after each sample to cleanse the palate. The characteristics of the evaluated samples were compared with those listed on a previously compiled table and points were awarded accordingly. A score of 5 points reflected the highest quality, while samples of the poorest (and unacceptable) quality were awarded 2 points. The acceptability threshold was established at 3 points.

2.2.3. Microbiological analyses

Microbiological analyses included determination of Total Viable Counts (TVC), *Enterobacteriaceae* family bacteria, Lactic Acid Bacteria (LAB), yeasts, total anaerobic counts and *Brochothrix thermosphacta*.

The total viable count was determined on plate count agar (PCA) incubated at 30° for 72 h in accordance with Polish Standard (PN-A-82055-6, 1994).

Enterobacteriaceae were determined using the plate counting method on Violet Red Bile Glucose agar (VRBG) as a medium after 24-hours' incubation at 37 °C (PN-A-04023:2001, 2001)

Yeasts were determined applying chloramphenicol medium with 5-day incubation at 25 ± 1 °C (PN-93 A-86034/07, 1993).

Determination of lactic acid bacteria was carried out on de Man, Rogosa, & Sharpe (MRS) medium after 72-hours' incubation at 30 °C (PN-ISO 15214, 2002).

Anaerobic forms were determined according to Babji and Murthy (2000) methodology. The PCA plates were incubated at 35 °C for 48 h in containers from which air was removed and replaced by nitrogen.

Detection of *B. thermosphacta* was conducted on Streptomycin Thallous Acetate Actidione (STAA) Agar Base. Incubation was performed for 48 h at 25 °C (Hayes, 1995).

2.2.4. Determination of pH

pH was determined using an HI 9025 (Hanna Instruments) pHmeter equipped with a combined electrode.

2.2.5. Determination of meat pigments

Myoglobin was extracted and quantified following the method described by Warriss (1979) and by Krzywicki (1982). Samples were homogenized with 0.04 M potassium phosphate buffer (pH = 6.80). Homogenates were held in an iced bath for 1 h to allow complete pigment extraction. Next, they were centrifuged (5000 *g*) for 30 min. at 0 °C. Supernatants were filtered twice through Whatman no.1 paper. Absorbance of clear supernatants was measured at 525, 545, 565 and 572 nm on a Lambda Bio + (Perkin Elmer) spectrophotometer. The total myoglobin content and the proportion of oxymyoglobin, myoglobin and metmyoglobin were calculated according to Krzywicki (1982).

2.2.6. Determination of amino nitrogen content

The content of amino nitrogen soluble in trichloroacetic acid (TCA) 5% solution was measured by the Pope and Stevens method, adapted by Fik and Surówka (1984).

2.2.7. Measurement of proteolytic activity

Total protease activity expressed as the amount of liberated tyrosine (μmol Tyr h^{-1} ml^{-1} extract) was determined by the autoproteolysis method described by Fik (1979). Samples were analysed under the conditions reported by Fik et al. (2008). Determination of tyrosine was carried out in accordance with the Anson method (Mejbaum-Katzenellenbogen & Mochnacka, 1969).

2.2.8. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis was carried out on fresh meat and on samples after 15 days' storage. Beef samples (3 g) were homogenized (Diax 900, Heidolph, Schwabach, Germany) for 2 min with twenty times their weight of deionised water. Then, aliquot (200 μl) of homogenate