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Studying the Possibilities of Using of Essential oils in Dairy Products. 2. Caraway (*Carum carvi L*)

KEYWORDS: caraway, essential oil, antimicrobial properties, lactic acid bacteria.

Iliana Kostova	Radka Vlaseva	Mihaela Ivanova
Ruse University „A. Kanchev", Branch-Razgrad, 47 Apr. Vastanie Blvd., 7200 Razgrad, Bulgaria	University of Food Technologies, 26 Maritsa Blvd., 4000 Plovdiv, Bulgaria	University of Food Technologies, 26 Maritsa Blvd., 4000 Plovdiv, Bulgaria

Stanka Damyanova	Nastia Ivanova	Albena Stoyanova
Ruse University „A. Kanchev", Branch-Razgrad, 47 Apr. Vastanie Blvd., 7200 Razgrad, Bulgaria	Ruse University „A. Kanchev", Branch-Razgrad, 47 Apr. Vastanie Blvd., 7200 Razgrad, Bulgaria	University of Food Technologies, 26 Maritsa Blvd., 4000 Plovdiv, Bulgaria

ABSTRACT *The possibility of using of the essential oil of caraway (Carum carvi L.) in dairy products has been studied. The composition, antimicrobial properties and its effect on the microorganisms of starter cultures for dairy products has been studied. It was found that it exhibits antimicrobial activity, but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. The essential oil of caraway is a suitable natural addition to dairy products.*

Introduction

The caraway (Carum carvi L.) is a biennial plant of the family Umbelliferae (Apiaceae), originating from Southeast Europe.

The fruits of caraway have strong scent and taste because of the main components of the essential oil – carvone and limonen. They are widely used spice in fats and hard-digested meals, sauces, soups, stewed potatoes, pickles, sausages, canned meat, in bakery products, cheeses, cottage cheese, etc., as well as in the alcohol- liqueur industry. In Germany and Austria fruits are added to sausages, pates, cheeses, different kinds of bread, dishes with cabbage, beet, meat, liqueurs and etc. (Boeva et al., 1990; Georgiev & Stoyanova, 2006; Hristov, 2008; Iacobellis et al., 2005; Petrovski, 2005).

The fruits are used in the phytotherapy, whole, powdered or as infusions. They are preferred spices because of their carminative, common- cordial and diuretic effect and they stimulate the appetite.

Very often they are used as detergent for kidney and biliary diseases. Furthermore, Romans and Indians used it to freshen the breath after eating. Tea made from the fruits is useful in colic. Essential oil, which has antimicrobial activity is used in medicine for flavoring drugs and preparations which have carminative, stimulating digestion and antispasmodic effect and stimulating the appetite (Agraharі & Singh, 2014; Ene et al., 2006; Georgiev & Stoyanova, 2006; Iacobellis et al., 2005).

The purpose of this work is to explore the possibilities for using of essential oil of the caraway fruits in the dairy products by examining its impact on the microorganisms from starter cultures for dairy products.

Experimental

Materials

Essential oil of fruits of caraway (Carum carvi L.) is used, obtained and analyzed by Damyanova et al. (2012)

1.1. Test microorganisms

To determine the antimicrobial activity of solutions of caraway oil are used test cultures from NBIMCC - National Bank of Industrial Microorganisms and Cell Cultures, Sofia: Gram-positive: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis ATCC 6633;* Gram-negative: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC *9027, Salmonella abony* NTCC 6017; Yeasts: *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231; Fungi: *Aspergillus niger* ATCC 16404, *Penicillium chrysogenum, Fusarium moniliforme.*

1.2.Starter cultures

Two starter cultures have been used for white brined cheese: mikroMILK TBMC1, contains: *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. lactis, Lactobacillus lactis subsp. cremoris, Lactobacillus casei;*

„LB Bulgaricum" JSC LBB CM 310-40 with composition: *Lactobacillus delbrueckii subsp.bulgaricus, Streptococcus thermophilus, Lactobacillus casei, Lactobacillus helveticus, Lactococcus lactis.*

2. Methods

2.1. Determination of the antimicrobial activity by agar diffusion method

The experiments were conducted on broth Soy- casein agar (Biolife) – for bacterias and Sabourad- dextrose agar (Biolife) for the yeasts and fungi. The oil of caraway was tested at concentrations of 100; 50; 10; 5; 1; 0,5; 0,1; 0,05 % Tween 80 in solution.

The diameters of the zones of the growth inhibition were measured in mm with a digital caliper, such as up to 15 mm microbial culture is less sensitive; from 15 to 25 mm – sensitive and over 25 mm – highly sensitive.

The experiments were conducted in parallel with controls from the solvent, taking into account and correct its effect.

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2.2. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC were determined according to the methods described by Andrews (2001), Barros et al. (2012) and Smith-Palmer et al. (1998).

2.3. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil of caraway on the microorganisms in starter cultures

The following concentrations of the oil were prepared: 1; 0,5; 0,1; 0,05 and 0,01 % in solvent sterile 1 % solution of Tween 80.

The growth of the bacterial cultures was assessed by comparing the number of the lactic acid bacterial in each suspension with that one of the control (bacteria suspension without oil). Inhibition of the growth of the lactic acid bacteria is read at that concentration of the essential oil in which is established 90 % reduction in the number of lactic acid bacteria compared to the control samples.

2.4. Studying of the influence of the caraway oil at concentrations suitable for the addition in food products on the microorganisms of the starter cultures

The studying is conducted according to the procedures for determining to the total number of lactic acid bacteria in starter cultures (Slavchev et al., 2000).

All results are presented as average of three parallel experiments.

Results and discussion

According to the content of the main components used the essential oil of caraway which is used by us does not distinguish from the data in the literature (Bourrel et al., 1995; Georgiev & Stoyanova, 2006; Sedlakova et al., 2000; Sedlakova et al., 2003). Among the identified 19 components the main (over 3%) are carvone and lemonen, the rest 17 are under 1% (Damyanova, 2012). It was found that the essential oil effects on all examined test microorganisms (Damyanova, 2012).

The results of the antimicrobial activity of solutions of the oil are presented in Figure 1.

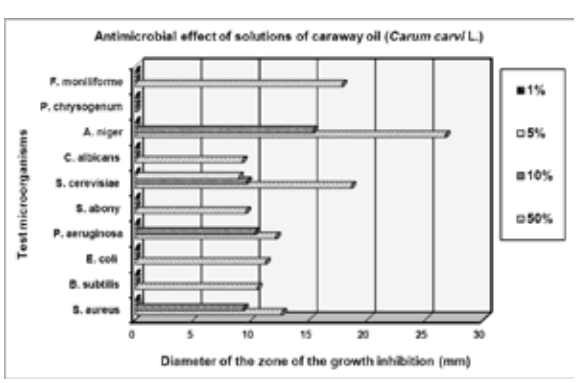

Figure 1. Antimicrobial activity of 50, 10, 5 and 1 % solutions of the caraway oil

The essential oil of caraway shows weak antibacterial activity of the 50% solution to Gram-positive and Gram-negative bacteria. It is highest in *Staphylococcus aureus* (12,6 mm zone of growth inhibition), *Pseudomonas aeruginosa* (12,1 mm zone of growth inhibition). 10% solution of caraway oil inhibits the growth only of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The solutions with concentration of 1% and 5 have not antibacterial activity.

The growth of *Candida albicans* is weakly inhibited by 50% solution of the caraway oil and is not affected by the solutions with concentration of 10, 5 and 1%. *Saccharomyces cerevisiae* is sensitive to the effect of 50% solution, less sensitive to 10 and 5% and insensitive to 1% solution of the essential oil of caraway.

From the tested fungi *Aspergillus niger* is high sensitive to the effect of 50% solution (26,7 mm zone of growth inhibition) and sensitive to 10% solution of caraway oil (15,3 mm zone of growth inhibition). The growth of *Fusarium moniliforme* is inhibited only by 50% solution of the oil. Our results for the antimicrobial activity of the essential oil of caraway correlate with data in the literature (Agrahari & Singh, 2014; Iacobellis et al., 2005; Seidler-Łożykowska et al., 2013).

To be able to add to the dairy products, the essential oil should not inhibit the development of the lactic acid bacteria which are contained in the dairy starters. In this regard, the influence of the caraway oil on the lactic acid bacteria from two starters. The obtained results are presented in Table 1.

Table 1. Influence of the solutions of essential oil of caraway (Carum carvi L.) on the growth of lactic acid bacteria in dairy starter cultures

Essential oil	**Concentration,** (µg/ml)	***Total number of viable colonies Lactobacillus sp,*** **(cfu/ml)**		***Total number of viable colonies S. thermophilus L. lactis, (cfu/ml)***	
		LBB CM 310-40	TBMC1	LBB CM 310-40	TBMC1
Colony	0	$6,7x10^7$	$2,6\ x10^7$	$1,1x10^8$	$1,2x10^8$
Caraway	0,01	$5,3x10^7$	$1,5x10^7$	$1,2x10^8$	$1,4x10^8$
	0,05	$4,3x10^3$	$3,2x10^5$	$4,7x10^5$	$5,5x10^4$
	0,5	0	0	0	0
	1,0	0	0	0	0

Data show that the growth of three kinds *Lactobacillus*, participating in the composition of the both starter cultures, is not affected by 0.01% solution of the essential oil. 0,05% solution of the oil vastly inhibits the growth of lactobacilli. The number of viable Colony Forming Units decreases strongly with the both starters. By increasing the concentration of the oil over 0.5% there is not a growth of *Lactobacillus sp.* Therefore MIC of the caraway oil against *Lactobacillus sp.*is 0.05%, and MBC is 0.5%.

The table also shows that the oil of caraway affects analogically the growth of *Streptococcus thermophilus* and *Lactococcus lactis* – 0,01% weakly inhibits their develop-

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ment, 0,05% vastly inhibits them and completely killing them over 0,5 %. Therefore MIC of the caraway oil against *Streptococcus thermophilus* and *Lactococcus lactis* is 0,05 %, and MBC is 0,5 %. Our results are confirmed by those of Mohamed et al (2013).

The addition of essential oils in food products requires an appropriate amount so as not to deteriorate their quality. On the other hand, they must not have depressing effect on the specific microorganisms, involved in the preparation of the fermented milk product. In this connection, the influence of the caraway oil is analyzed at concentrations 0,0008, 0,002 and 0,003%, which can be used in food products (Georgiev & Stoyanova, 2006). The obtained results are shown in Table 2.

Table 2. Effect of the essential oil of caraway in dosages suitable for foods products on lactic acid bacteria in dairy starter

Essential oil	Concentration, (%)	*Total number of viable colonies Lactobacillus sp,* (cfu/ml)		*Total number of viable colonies S. thermophilus* L. lactis, (cfu/ml)	
		LBB CM 310-40	TBMC1	LBB CM 310-40	TBMC1
Control	0	$6,7x10^7$	$2,6 x10^7$	$1,1x10^8$	$1,2x10^8$
Caraway	0,0008	$4,3x10^7$	$2,5x10^7$	$1,4x10^8$	$1,1x10^8$
	0,002	$3,4x10^7$	$2,3x10^7$	$1,0x10^8$	$1,3x10^8$
	0,003	$3,4x10^7$	$2,1x10^7$	$1,1x10^8$	$1,2x10^8$

The growth of *Lactobacillus* in the two starter cultures used is not affected by the presence of the essential oil of caraway in concentrations which can be used in food products. The number of colony forming units with the addition of essential oil is approximately equal to the number of the colonies in the control samples. *S. thermophilus and L. lactis* is also not sensitive to the used concentrations of the caraway oil in the tested starters: LBB CM 310-40 and TBMC1.

The used amounts of the caraway oil do not negatively affect the development of lactic acid bacteria in the studied starter cultures.

Conclusion

The essential oil of the fruits of caraway exhibits antimicrobial activity but does not inhibit the development of the lactic acid bacteria in dairy starter cultures. Minimum inhibitory concentration of the caraway oil against *Lactobacillus sp., Lactococcus lactis* and *Streptococcus thermophilus* is 0,05 %, and minimum bactericidal concentration is 0,5%. The essential oil of caraway is suitable natural addition to dairy products.

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