PRELIMINARY EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF SOME SPICES USED AS ADDITIVES IN TOMATO SAUCE PRODUCTS

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The aim of this work was preliminary evaluation of the antimicrobial activity of several additives that can be used in tomato sauce products. For this purpose, several frequently used spices and the food supplement monolaurin were applied in the process of the tomato sauce production. The spices used were: garlic powder, basil leaves, cinnamon powder, milled chili peppers and table salt. Several different wild types of fungi were isolated from the rotten tomato and rotten apricot and the latter one was used as an infecting microorganism for tomato sauce products. The tests of the samples of the infected tomato sauces containing monolaurin, basil and cinnamon as additives had total cell counts of 3 ± 1 , 8 ± 1 and 6 ± 1 cfu/mLx10⁶. Those results showed that the three selected additives are characterized with the highest potential to be used as antimicrobial additives in the tomato sauce production process.

Keywords: tomato sauce, monolaurin, basil, cinnamon, garlic, chilli peppers, antimicrobial activity

Introduction

The tomato plant (*Solanum lycopersicum* L.) is one of the most frequently produced agricultural species that is readily consumed worldwide as both fresh and processed food product. The ten largest producers of tomato products are: the USA, China, Italy, Turkey, Spain, Brazil, Portugal, Greece and Chile [1]. The calculation of its nutritional value, performed on the basis of the tomato sauce product, shows that the tomato contains a wide spectrum of nutritionally valuable compounds such as lycopene (71.6%), vitamin C (12%), β -caroten (17.2%) and vitamin E (6%) [2]. It is also very rich in potassium (237 mg/100 g tomato), while having a very low caloric value (3.89 g carbohydrates and 0.2 g fat/100 g tomato).

The common procedure for the industrial production of the tomato sauce assumes application of a vacuum evaporator. Both preservation and adjustment of a certain dry mass content might be achieved by using this technique. Besides common thermal procedures for the preservation of food products and conventionally used chemical preservation techniques, the unconventional techniques such as the addition of spices as natural preserving agents having antimicrobial activity are also widely investigated nowadays [3]. Natural antimicrobials such as essential oils of cinnamon [4-5], extracts of garlic [6-7], extracts of neem [8], basil [9, 10] and other spices, oleoresins, fatty acids and their esters are widely used in different food products in order to not only establish a safe food preservation process, but also give the product a new quality and the added value. Thus, monolaurin and other partial glycerides of fatty esters are well known for their pronounced antimicrobial activity, expressed both "in vitro" or as an additive incorporated in some kind of a food product [11-14]. While investigating the antimicrobial effect of monolaurin, Kabara and Marshal [15] found that this partial glyceride strengthens

the immune system, and that its protective effect is evident during its consumption and sometime afterwards. The modern food technology should at the same time guarantee food safety and the preservation of nutritionally and biologically valuable compounds [3]. The last techniques are considered to be of a special interest today, when the struggle for the consumption of healthy food is especially expressed among the well informed consumers. Thus, the preservation of food products by using natural antimicrobials is a very important research area of the modern food technology.

Regarding the safety of tomato sauces, there are data that confirm that several moulds and bacterial species might be the cause of microbial spoilage of those food products [16].

In this work, several wild microbial strains were isolated from rotten fruits and vegetables. The isolated cultures were purified and morphologically analysed. The produced tomato sauces, all having different spices and monolaurin, were infected by the isolated wild microbial strain. A preliminary evaluation of the antimicrobial effect of the used spices and monolaurin was performed.

Material and methods

Material

Monolaurin which was used was in a form of capsules containing the powder with 99% of purity produced by „Ecological formulas“, Concord, California, the USA.

The microbiological safety of tomato sauces was analysed by using Sabouraud agar medium. It was a product of Sigma-Aldrich, Buchs, Switzerland.

Two wild fungal strains were used as test microorganisms i.e. as infecting microorganisms of tomato products. The fungal strains (grey and green mould) were isolated from the

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rotten apricot and rotten tomato. The cultures were purified. Since the first one was more resistant to infections and had the more pronounced growth rate and concentration of cells ($1 \times 10^9/\text{mL}$), it was chosen for further experiments.

The spices: chilli peppers (*Capsicum annuum*), basil leaves (*Ocimum basilicum*), cinnamon (*Cinnamomum cassia*), garlic (*Allium sativum*) and the raw material for the food products, tomatoes, were all bought from the local food store.

Methods

Preparation of the tomato sauce

Tomatoes were first blanching for 8 minutes at the temperature of 88°C , milled, and then the water content was adjusted by a vacuum evaporation until the dry mass of 8.5% and a final volume of 340 mL were adjusted (Figure 1.1). Chilli peppers, basil leaves, cinnamon, garlic, salt and monolaurin were all used as additives in tomato sauce products. Monolaurin was added in the concentration of 0.2% (w/v) and all other additives were added in the concentration of 0.5% (w/v).

Figure 1. Tomato sauces produced in the Laboratory for Food Technology and Biotechnology, at the Faculty of Technology and Metallurgy, Skopje (1), and the counting of the colony forming units from the infected samples on a Petri Dish (2)

Isolation of the microorganisms

Several wild types of microorganisms were isolated from the rotten apricot and rotten tomato samples. The microorganisms were purified by a technique of repeated dilution and incubation, until a single cell colony was grown from each and every culture. The isolated strains were morphologically examined.

Infection of the tomato products

The tomato sauce was infected by 3 mL suspension containing 1×10^9 cells/mL. The infected tomato sauce was kept at 30°C for 72 h.

Preparation of the growth medium

The Sabourand dextrose agar was prepared and autoclaved at 121°C for 35 minutes. After cooling, the medium was dosed aseptically in 12 mL quantities into Petri Dishes (150x25 mm).

Microbiological tests

From all different tomato products, the 25 mL sample was taken and diluted with 225 mL sterile water. Then further dilutions of 10^{-5} , 10^{-6} and 10^{-7} were prepared. 1 mL of each dilution was taken and inoculated on a Petri Dish with 12 mL Sabourand agar. Thus inoculated Petri Dishes were incubated on 30°C for 4 days. Then, the number of the colonies grown from a single cell (colony forming unit) was counted (Figure 1.2).

Figure 2. The wild green mold (1) isolated from the rotten apricot (2) in the Laboratory for Food Technology and Biotechnology, at the Faculty of Technology and Metallurgy, Skopje.

Results and discussion

Isolation of wild type plant infecting microorganisms
Several wild types of microbial strains were isolated from rotten tomatoes and rotten apricot. Moulds can easily grow at relatively low pH values, so the tomato sauce having pH around 4 is an excellent medium for the fungal growth. The growth of the fungus isolated from the rotten apricot is presented in Figure 2 (2.1 and 2.2). The microorganism grows very quickly and intensively on the Sabourand-dextrose solid medium in the Petri Dish (Figure 2.1).

Figure 3. Microscopic appearance of the moulds isolated from: 1 (tomato-green mould), 2 (tomato-grey mould), 3 (apricot-green mould), 400 times enlarged

The isolated fungi were purified by multiple dilution and the one isolated from the rotten apricot has been chosen as an infecting strain due to its robustness, resistance to infections of other microbial strains and a high density of growth. The isolated fungal strains were also microscopically examined. The results are presented in Figure 3. It is interesting to note that in Figure 3.3, the hyphal structure of the microorganism can be recognized.

Figure 4. The growth of the fungus isolated from tomato sauce containing different additives: control, chili peppers, monolaurin and basil. The dilutions of the samples used were 10^{-7} , 10^{-6} and 10^{-5}

Microbiological tests of the infected tomato products

The fungus presented as the fungus number 3 *i.e.* the apricot green mould was selected as an infecting microorganism for tomato sauces containing different food additives as antimicrobial agents. After the infection of different sauces (3 mL with the concentration of 1×10^9 cells/mL) and incubation, the samples of 25 mL were taken and grown colonies were counted according to the procedure described in the M&M section. The appearance of the grown fungus, from the samples prepared with dilu-

tions of 10^{-7} , 10^{-6} , 10^{-5} is presented in Figure 4 and the total cell counts of the colonies formed are presented in Table 1.

From the presented results, it is obvious that monolaurin, cinnamon and basil pronounced the antifungal activity. That monolaurin affects both vegetative cells and reproductive organelles of the fungus, has already been evaluated [17]. Kabara and Marshall [15] also claimed that monolaurin, as an antimicrobial agent having natural origin, is characterised by the antimicrobial activity not only *in vitro*, but its strong antimicrobial effect continues after its consumption in the human organism, and in this way it strengthens the immune system of the body. Thus, these kinds of food products that contain biologically active agents are considered as nutraceuticals, i.e. functional food products.

Table 1. Preliminary determination of the antimicrobial activity of additives used in the tomato sauce production, according to the total cell count of the fungus

Tomato sauce type	Total cell count (cfu/mLx10 ⁶)
Monolaurin	3±1
Basil	8±1
Chilli peppers	10±5
Salt	15±5
Control	15±5
Cinnamon	6±1
Garlic	15±5

Basil leaves, used as spices in the tomato sauce have also shown a strong antifungal activity (total cell count of 8 ± 1 cfu/mLx10⁶). That the essential oil from basil leaves, one of the most frequently used spices in the food production industry are well known to have a broad range of antimicrobial activities towards various gram positive bacteria (*Streptococcus*, *Staphylococcus*), gram positive bacteria (*Escherichia*, *Pseudomonas*) and fungi (*Aspergillus*, *Penicillium*), has already been reported [9].

The antifungal activity of cinnamon, as a spice used in tomato products, has also a distinguished value (6 ± 1 cfu/mLx10⁶). According to the literature, this very special spice is very rich in essential oils with a strong antimicrobial effect towards wide varieties of microbial strains [4, 5]. There are opinions that these essential oils negatively affect the cell replication mechanism and inhibit the activity of the enzymes responsible for the replication of microbial cells, which is a probable explanation for their strong antimicrobial effect [5]. The use of salt and garlic as additives failed to show any antimicrobial effect, while chilli peppers showed a very mild antimicrobial effect towards the examined wild fungal strain.

Conclusions

Several food additives (spices) were used in the model tomato sauce production with preliminary evaluation of their antimicrobial activity. The basil leaves, cinnamon powder, garlic powder, salt and milled chilli peppers as spices, and monolaurin as a food supplement were all used as additives of the produced tomato sauces. Monolaurin, basil and cinnamon showed the strongest antifungal activity, the property that can be used in the creation of the preservation process which is safe but less harmful for biologically active compounds.

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Izvod

PRELIMINARNO ISPITIVANJE ANTIMIKROBNE AKTIVNOSTI POJEDINIHZAČINA KOJI SE KORISTE KAO ADITIVI U PROIZVODNJI SOSOVA OD PARADAJZA

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Cilj ovog rada je preliminarno ispitivanje antimikrobne aktivnosti različitih aditiva koji se mogu koristiti u proizvodnji različitih sosova od paradajza. Za ovu namenu, u procesu proizvodnje paradajz sosa korišćeno je nekoliko različitih začina i monolaurin kao dijetetski dodatak ishrani. Začini koji su upotrebljeni su: beli luk u prahu, listovi bosiljka, cimet u prahu, mlevena, ljuta, crvena paprika i kuhinjska so. Nekoliko različitih vrsta divljih gljiva bile su izolovane od trulih paradajza i trulih kajsija. Ova druga gljiva, izolovana od trule kajsije, bila je korišćena kao infektivni mikroorganizam za pripremljene paradajz sosove. Testovi primeraka inficiranih paradajz sosova koji kao additive sadrže monolaurin, bosiljak i cimet imali su ukupan broj ćelija od 3 ± 1 , 8 ± 1 i 6 ± 1 cfu/mLx10⁶. Ovi rezultati su pokazali da se tri odabrana aditiva karakterišu sa najvećim potencijalom za upotrebu kao antimikrobni aditivi u procesu proizvodnje paradajz sosova.

Ključne reči: paradajz sos, monolaurin, bosiljak, cimet, luk, ljute paprika, antimikrobna aktivnost