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Research Article

# The influence of dark chocolate consumption on the microbial population in the oral cavity

**Mișcă Corina[1], Raba Diana[2], Dumbravă Delia[1]*, Moldovan Camelia[1], Borozan Aurica Breica[3]*, Popa Mirela[1], Rinovetz Alexandru[1] and David Ioan[1]**

[1]Faculty of Food Engineering, University of Life Sciences 'King Mihai I" from Timișoara, Romania
[2]Faculty of Management and Rural Tourism, University of Life Sciences 'King Mihai I" from Timișoara, Romania
[3]Faculty of Horticulture, University of Life Sciences 'King Mihai I" from Timișoara, Romania

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***Corresponding author:** Dumbravă Delia, Faculty of Food Engineering, University of Life Sciences 'King Mihai I" from Timișoara, Romania, Tel: +40740151601; E-mail: deliadumbrava@usab-tm.ro

Borozan Aurica Breica, Faculty of Horticulture, University of Life Sciences 'King Mihai I" from Timișoara, Romania, Tel: +40724704366; E-mail: auricaborozan@usab-tm.ro

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## Summary

This study was initiated at the Faculty of Food Engineering within the Mihai I University of Life Sciences in Timișoara, with the aim of identifying the effect of dark chocolate with a high concentration of cocoa on the microbial population in the oral cavity, which facilitates the formation of dental plaque, tartar and implicitly the appearance of dental caries. The specialized literature directed us to this study and we wanted to highlight the presence of microorganisms that can be isolated from the oral cavity and the effectiveness of this treatment - the consumption of dark chocolate after meals, in small quantities. The methods for isolating microorganisms were the classic ones imposed by the standards. The results obtained confirmed the bactericidal effect of dark chocolate with a high cocoa content. In conclusion, the consumption of a quantity of 10 grams of dark chocolate, after the meal exerted a beneficial effect on oral hygiene, by reducing the number of acidogenic microorganisms.

## Introduction

The oral environment is a physicochemical ecosystem that occupies and influences the structures of the oral cavity and includes transitional elements, represented by food and air, provisional own elements, represented by saliva and the mobile and fixed microbial flora from the dental plaque, dental tartar, and saliva as well as own, fixed components: teeth, tongue, oral mucosa. All these structures are in permanent interaction, and fluctuations in the composition of the environment have direct repercussions on the fixed components. Salivary pH is approximately 6.7 at rest, with variations between 5.2-7.6. It shows variations depending on the type of secretion, age, meal times, time of day, and salivary flow. Salivary pH depends on blood carbon dioxide concentration, diet, and salivary buffer systems: bicarbonate - carbonic acid, disodium phosphate - monosodium phosphate, and the buffer capacity of saliva shows diurnal variations, especially according to diet. Dental plaque is a soft, adherent biofilm, colored or not, located on the surface of the tooth, with an important role in the occurrence of caries. The community of microorganisms, attached to the surface of the teeth, is organized in a three-dimensional structure and anchored on a matrix of extracellular materials of both bacterial and salivary origin. This matrix has an important role in the overall strength and structural integrity of the formed biofilm [1]. Matrix development takes about 24 hours. During 2-4 days after the formation of the matrix, colonies of microorganisms appear on its surface, belonging to different microbial genera and species. The microorganism most frequently encountered at the level of dental plaque is *Streptococcus mutants*, being considered the most frequent etiological agent of dental caries. *S. mutans* produces enzymes - glucosyltransferases - with a role in the conversion of sucrose into extracellular homopolymers of glucose - glucans, which provide the adhesive skeletal substrate on which the biofilm is anchored [2,3]. The microorganism also synthesizes extracellular fucosyltransferase that converts



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sucrose into fructooligosaccharides, which rapidly accumulate in oral biofilms serving as storage polymers. Their metabolism by a fructosidase of microbial origin increases the amount and duration of acid production and thus, the virulence of the organism [4,5]. A special concern is given at this time to nutrition, in order to prevent the formation of dental plaque and the appearance of dental caries. In this sense, Addai, et al. [6] analyzed, among other foods, different varieties of chocolate for their acidogenic effects on teeth. They found that one variety of milk chocolate produced in Ghana, which contained 30% cocoa solids, was not acidogenic [6]. There is research carried out by Paolino and Kashket since 1985, that highlights the beneficial effect of cocoa powder, inhibiting the accumulation of dental plaque and the formation of caries by reducing the production of polysaccharides and preventing periodontal diseases.

In this study we tried to highlight the effect that dark chocolate consumption has on the microbial population in the oral cavity [7]. Considering the current tendency to pay special attention to their career, lunch and often dinner are meals served in the city, which is why brushing their teeth consecutively is impossible. That is why we set out to highlight the antibacterial effect of dark chocolate with 72% cocoa content, which could be consumed after meals, in small quantities, and the results would be beneficial in terms of oral hygiene.

## Materials and methods

The aim of this work was to determine the effect of consuming dark chocolate with a content of more than 70% cocoa, on the bacterial strains in the oral cavity. The objectives of this study were: the isolation and identification of all bacterial strains isolated from the oral cavity of 15 volunteers, students of the Faculty of Food Engineering, and future technological engineers, as well as determining the bactericidal effect of black chocolate on the isolated strains.

Between April 2023 and May 2023, we collected biological samples from the oral cavity of the volunteers using the exudate method. The collection of samples to obtain the inoculum, in order to isolate the microorganisms subjected to microbiological investigations, followed the ethical laboratory guidelines and written informed consent was obtained from each evaluator in accordance with the European Union guidelines on ethics and research in the food field [8]. The processing of biological products was carried out in the microbiology laboratory of the Faculty of Food Engineering. The isolated strains were identified using the gallery cards API - Staph, API - Strepto, Api 20 E for enterobacteria, API 20 NE for nonfermentative Gram-negative germs, and Saliva - Check mutans. Sensitivity testing for anti-infective chemotherapeutics was performed according to the standardized Kirby-Bauer diffusometric method. The reading and interpretation of the results were carried out according to the CLSI 2022 international standards [9]. The method used for chocolate microbial susceptibility testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and with minimal adjustments based on our previous studies [10]. The microbial suspension was prepared at 0.5 McFarland using a standard saline solution for each strain and inoculated onto Mueller-Hinton agar (bioMérieux, Marcy-l'Etoile, France). Subsequently, a disk (BioMaxima, Lublin, Poland) containing 10 µL of chocolate suspension was to be tested and disks containing 5 µg levofloxacin for positive control were placed on these plates. Zones of inhibition were measured in millimeters after a 24-hour incubation at 35 - 37 C, for the bacterial species used. All tests were performed in triplicate. The hemolytic activity of the microorganisms was tested in Petri dishes on the agar-blood medium. Bacterial morphological types were highlighted on microscope slides using Gram staining.

## Results

Over a period of one month, we isolated a consistent number of microorganisms belonging to different morphological types – isolated cocci, diplococci, sarcinae, streptococci, staphylococci, and bacilli. Through the specific methods of isolation and identification, we highlighted the presence of germs belonging to the genera: *Staphylococcus aureus, Streptococcus mutans, Streptococcus pyogenes, Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumoniae*, staphylococci, non-hemolytic streptococci, but also gram-positive bacilli, but also gram-negative bacilli and coccobacilli. The volunteers from whom the collection was made were young people between the ages of 19 and 28, and the gender distribution was 9 girls and 6 boys. All were healthy young people with no pathological complaints. Although the microbiological picture identified in the isolates from the oral cavities of young people, highlights the presence of commensal bacteria in the majority, we cannot ignore the presence of opportunistic germs with pathological significance. Thus, the presence of the *Staphylococcus aureus* germ in the upper digestive segment, without being accompanied by a beta-hemolytic streptococcus, does not present signs of concern. At the same time, we cannot ignore the fact that we never have a monobacterial contamination, but always a mixed contamination, which can be considered an indicator of the state of oral hygiene. Given today's chaotic diet, we considered this study timely and wanted to check how eating chocolate that contains a higher amount of cocoa could help us maintain a higher state of oral health. It is well known that during the day, after meals, we cannot achieve proper oral hygiene, by brushing our teeth, because those are the optimal conditions, which is why we should consume foods that would reduce the acidity of the oral cavity. Also based on previous studies, we decided to check the effect of dark chocolate on microorganisms isolated from the oral cavity [11,12]. In this way, the microorganisms collected from the surface of the teeth were isolated and cultivated by specific methods, and then the effectiveness of the dark chocolate suspension, which contains 72% cocoa, was tested on these microorganisms cultivated in Petri dishes, on Muller Hinton culture medium, compared to the effectiveness of the antibiotic levofloxacin. The antimicrobial activity of the chocolate suspension on the tested microorganisms was evaluated by measuring the zone of inhibition around the micro tablet containing 10 µl of chocolate suspension, obtained by dissolving 10 grams of chocolate in 10 ml of water. The obtained results are listed in Table 1.



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**Table 1:** The antimicrobial activity of the chocolate suspension, highlighted by the disk diffusion method, on the analyzed microorganisms.

| Bacterial strains | Disk diffusion (mm) Chocolate suspension | Disk diffusion (mm) Levofloxacine 5 µg |
|---|---|---|
| Staphylococcus aureus | 19 ± 26 | 25 ± 0,14 |
| Streptococcus mutans | 21 ± 0,23 | 22 ± 0,10 |
| Streptococcus pyogenes | 20 ± 0,16 | 23 ± 0,23 |
| Serratia marcescens | 20 ± 0,10 | 25 ± 0,10 |
| Pseudomonas aeruginosa | 8 ± 0,10 | 24 ± 0,17 |
| Klebsiella pneumoniae | 15 ± 0,23 | 23 ± 0,26 |

The antibacterial activity of the chocolate suspension is tested in a comparative way with the effect exerted by levofloxacin on the tested microorganisms, and the results are expressed in mm and include the size of the filter paper disk, which is 6 mm. The results are presented as mean ± Standard Deviation (SD) of the zone of inhibition.

Analyzing the data from the tables, we noticed that the effects of the chocolate suspension with a 72% cocoa content on the analyzed microorganisms vary within quite wide limits, from a halo size of 8 mm, in the case of the *Pseudomonas* sp germ, for which we can appreciate that it has no inhibitory effect, up to 21 mm size, in the case of the germ *Streptococcus mutans*, which germ is practical, the most incriminated, in the production of dental plaque and dental caries. With the exception of the Pseudomonas aeruginosa germ, which has no bacteriostatic or bactericidal effects, the other microorganisms tested are strongly inhibited by the dark chocolate suspension, which is why we can state that in fact, eating a chocolate square of approximately 10 grams after a meal could reduce the acidogenic effect of the food consumed.

Considering the data in the table, we can observe the susceptibility of the tested microorganisms, in the following order: *Streptococcus mutans, Streptococcus pyogenes, Serratia marcescens, Staphylococcus aureus, Klebsiella pneumoniae*, the difference in susceptibility to some strains, compared to the others, could be put on the ability of the chocolate suspension to penetrate the parietal structure and probably the membrane structures of some of the bacterial cells, disrupting the chemiosmotic control, with a lethal effect on the bacterial structures [13-15].

In many situations, chewing gum is consumed after a meal. Between consuming chewing gum and a 10-gram piece of dark chocolate, we advocate for the second option, which we have proven to have a bactericidal effect on many of the microorganisms found in the oral cavity.

## Conclusion

Dark chocolate with a high cocoa content, over 70%, exerts beneficial effects on oral hygiene, through the bactericidal effects on many microorganisms in the oral cavity, which contribute to the formation of dental plaque and implicitly to the appearance of dental caries. Chocolate is a product with high energy value, with highly appreciated organoleptic properties, recommended to be consumed in moderation, especially the one that contains a high percentage of cocoa and less, milk chocolate. Considering the bactericidal effect, it is recommended that after the meal, when there is no possibility to brush the teeth, consume a piece of 10 grams of dark chocolate with an important effect of reducing the action of bacteria with an acidogenic effect, which affects the dental enamel, facilitates the appearance of caries and their rapid multiplication also leads to the appearance of oral halitosis.

**Institutional review board statement:** The study was carried out in compliance with the Declaration of Helsinki and approved by the Bioethics Committee of the University of Life Sciences "King Michael I", Timisoara, Aradului Street No 119, 300645 Timisoara, Romania (No 206/04 April 2023); Project Code: POR/2020/1/1.1.A./2/140030.

**Informed consent statement:** Informed consent was obtained from all subjects involved in the study.

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