

Annex no. 2 H.S. no. 160/17740/26.07.2023

**“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND  
PHARMACY TIMIȘOARA  
FACULTY OF DENTAL MEDICINE  
DEPARTMENT I**

**POPESCU D. LIVIANA- ALEXANDRA**



# **DOCTORATE THESIS**

**MODERN TECHNIQUES IN DENTAL  
PROPHYLAXIS**

**ABSTRACT**

Doctorate Coordinator

**UNIV. PROF. DR. SINESCU COSMIN**

**Timișoara**

**2024**

## TABLE OF CONTENTS

<b>LIST OF PUBLISHED SCIENTIFIC PAPERS .....</b>	<b>VI</b>
<b>LIST OF ABBREVIATIONS AND SYMBOLS.....</b>	<b>VII</b>
<b>LIST OF FIGURES .....</b>	<b>IX</b>
<b>LIST OF TABLES.....</b>	<b>XIV</b>
<b>DEDICATION (optional) .....</b>	<b>XVII</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>XVIII</b>
<b>INTRODUCTION .....</b>	<b>1</b>
<b>GENERAL PART .....</b>	<b>5</b>
<b>1. NUTRITION, RISK FACTOR IN ORAL HEALTH ..</b>	<b>5</b>
1.1 INTRODUCTION .....	5
1.2. DENTAL CARIES .....	6
1.2.1 WORLDWIDE PREVENTION OF DENTAL CARIES .....	6
1.2.2 ETIOLOGY OF DENTAL CARIES .....	7
1.3. GENERAL HEALTH HARMFUL EFFECTS OF A CARIOGENIC DIET .....	9
1.4. PROPHYLAXY OF DENTAL CARIES BY THE CORRECT MANAGEMENT OF THE DENTAL CARIES .....	9
<b>2. SPECIES OF PLANTS WITH ANTIOXIDANT, ANTIBACTERIAL AND ANTICARIOGENIC PROPERTIES.....</b>	<b>10</b>
2.1. NONCARIOGENIC NATURAL SWEETENERS .....	10
2.2. BENEFICIAL PLANT PROPERTIES .....	11
<b>3. HOLISTIC DENTAL MEDICINE.....</b>	<b>17</b>
3.1. TRADITIONAL DENTAL PREVENTION .....	17

3.2. HOLISTIC DENTAL PREVENTION.....	18
3.3 NATURAL ORAL CARE PRODUCTS USED IN DENTAL MEDICINE .....	19
<b>SPECIAL PART .....</b>	<b>22</b>
<b>4. EVALUATION STUDY OF THE INTERACTION BETWEEN THEOBROMINE IN COFFEE BEANS AND SWEETENERS .....</b>	<b>23</b>
4.1. INTRODUCTION .....	23
4.2. AIM AND SPECIFIC OBJECTIVES OF THE STUDY .....	23
4.3. MATERIAL AND METHOD.....	23
4.4 RESULTS .....	27
4.4.1. FTIR-UATR SPECTRUM.....	27
4.4.2 THERMOGRAVIMETRIC ANALYSIS .....	29
4.5. DISCUSSIONS .....	38
4.6 CONCLUSIONS.....	41
<b>5. COMPARATIVE SENSORIAL EVALUATION STUDY BETWEEN THE FIRST HOT CHOCOLATE PRODUCT AND A REFERENCE COMMERCIALY AVAILABLE PRODUCT .....</b>	<b>43</b>
5.1. Introduction .....	43
5.2. Aim and specific objectives of sensorial evaluation.....	43
5.3. Material and Method .....	44
5.4 Results and Discussions.....	48
5.5 Conclusions .....	62

<b>6. COMPARATIVE STUDY FOR THE SENSORIAL EVALUATION OF A NEW OPTIMIZED HOT CHOCOLATE PRODUCT AND THE SAME REFERENCE COMMERCIALY AVAILABLE PRODUCT .....</b>	<b>63</b>
6.1. Introduction .....	63
6.2. Aim and objectives of the second evaluation.....	63
6.3. Material and Method .....	64
6.4. Results and Discussions.....	69
6.5 Conclusions .....	80
<b>7. EVALUATION STUDY OF THE ANTIOXIDANT, ANTIMICROBIAL AND TOXICOLOGICAL EFFECTS OF THE TWO TYPES OF SUGARFREE VEGAN HOT CHOCOLATE .....</b>	<b>81</b>
7.1. Introduction .....	81
7.2. Aim and specific objectives of the study .....	81
7.3. Material and method .....	82
7.3.1. EVALUATION OF THE TOTAL POLYPHENOL CONTENT BY THE FOLIN –CIOCÂLTEAU METHOD ().....	83
7.3.2.EVALUATION OF THE INDIVIDUAL POLYPHENOLS BY LIQUID CHROMATOGRAPHY () .....	83
7.3.3. EVALUATION OF THE ANTIOXIDANT CAPACITY (AC) BY THE CUPRIC REDUCING ANTIOXIDANT CAPACITY (CUPRAC) () .....	84
7.3.4 EVALUATION OF THE PROXIMATE COMPOSITION, NUTRITIONAL PROFILE AND ENERGETIC VALUE ( .....	84
7.3.5. EVALUATION OF THE ANTIMICROBIAL ACTIVITY ().....	84
7.3.6. THE IN VITRO TOXICITY TEST ().....	85

7.3.6.1 Culture of Cell Lines .....	85
7.3.6.2. The Alamar Blue test for cell viability .....	86
7.3.6.3. Sensorial evaluation .....	86
7.3. Results and Discussions .....	87
4.5. Conclusions .....	107
<b>GENERAL CONCLUSIONS .....</b>	<b>109</b>
<b>REFERENCES .....</b>	<b>113</b>
<b>ARTICLES PUBLISHED <i>IN EXTENSO</i> .....</b>	<b>II</b>

## INTRODUCTION

The presented doctorate thesis entitled MODERN TECHNIQUES IN DENTAL PROPHYLAXIS is based on the wish to develop a food product exclusively from plants with major benefits for the health of the individual due to the potential of plants to exert antimicrobial, anticariogenic, antioxidant and antidiabetic effects; designing such a food product with a major anticariogenic effect represents an essential milestone in the development of dental prevention but also for maintaining the health of the entire organism.

Most prevention products do not include food products. This situation generates a great opportunity to develop research in this direction, as carious lesions affect most of the population worldwide. The consumed sweets do not only affect teeth but also favour diabetes and obesity nowadays already widely occurring at young ages. This led to the conclusion that, nowadays, a food product that does not favour carious lesions and also does not affect the body mass index and glucose serum levels, even in case of increased intake, is a high necessity.

Over time, various plant species have been used for manufacturing oral hygiene and, also, food products with important roles in maintaining oral and general health, due to their multiple benefits. By enhancing the use of plants, a novel vision and a different approach to prevention manoeuvres emerged, diversified in comparison with traditional, well-known prevention methods.

The discovery of plant combinations that might be regarded as ingredients of natural sugar-free, highly antioxidant, antibacterial and

anticariogenic food products is of high interest for developing the holistic domain in dental medicine.

This doctorate thesis offers vast and detailed research on the modern prevention methods of dental diseases, with a special accent on the use of medicinal plants and the impact of nutrition. It is structured in several chapters that cover theoretical and practical aspects of dental prophylaxis.

## **GENERAL PART**

The general part includes three chapters that analyze the relevant scientific literature. These chapters are essential to understanding the significance of the studies conducted in the special part section of this doctorate thesis.

**The first chapter** of this part represents an introduction that provides data regarding the prevalence of caries and the main risk factors, as oral diseases are perceived as one of the major problems affecting the world's population. There is a special accent on the cariogenic diet and the negative effects of sugar on teeth, but also the prevention of dental caries by correct diet management. The relationship between diet and oral health is important because oral health represents an important life quality and welfare indicator.

**The second chapter** offers a comprehensive synthesis of the benefits of plants in various areas of health and nutrition. This chapter serves as an essential guide for understanding the contribution of plants, by their bioactive components, to the prevention and treatment of various diseases. In other words, this chapter offers a detailed perspective on the natural plant species representing a healthy alternative to chemical synthetic products.

**The third chapter** of the general part starts by providing a solid basis for traditional dental prevention that remains a central element of oral health. The detailed description of the traditional prevention methods establishes a necessary starting point for understanding the need for a holistic approach. Holistic prevention in dental medicine includes the use of natural remedies and plant-based products that are safe and effective for the treatment and prevention of oral diseases. The holistic prevention in dental medicine brings a comprehensive and deep perspective on oral health, underlining the interconnection between oral and general health. By promoting adequate nutrition, avoiding toxins, using natural remedies, and non-invasive approaches and treating deep causes, holistic prevention offers a safe and efficient way to maintain and improve dental health.

## **THE SPECIAL PART**

The special part is structured into four chapters with the following objectives: to detect the biocompatibility of theobromine in cocoa beans with various sweeteners such as sorbitol, trehalose, fructose, galactose, glucose, gum Arabic and lactose to obtain a food product with numerous general health benefits; to design and produce a hot chocolate recipe meant to combine the taste, flavour, texture and smell for an antioxidant and anticariogenic food product and last but not least to determine the in vitro toxicologic profile, nutrition properties, total and individual content of polyphenols and the anticariogenic, antioxidant, anti-carcinogenic and antimicrobial activities in the case of the two new, vegan and sugar-free chocolate products.

**The first study** of the doctorate thesis, described in **the fourth chapter**, offers detailed research on the interaction of theobromine



with various sweeteners under the influence of high temperatures. This study aims to identify the optimal combinations usable in natural and antioxidant dental products. The study includes a detailed methodology using ATR-FTIR spectroscopy (Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy). This technique has been used to observe the molecular interactions in chemical bonds. It allows the detection of infrared absorption spectra changes that indicate the presence or absence of chemical interactions between theobromine and sweeteners. Thermal Analysis (TG/DTG/HF-Thermogravimetric Analysis/Differential Thermogravimetric/Heat Flux Analysis) has also been used. The thermogravimetric and heat flow analysis techniques have been used to study the thermic stability of samples and to identify the melting and thermic degradation points. The sweeteners used to study theobromine-sweetener interactions were as follows: sorbitol, trehalose, fructose, galactose and glucose, gum Arabica and lactose. In the case of thermogravimetric analysis, this study used the TG/DTA Diamond Perkin Elmer (Diamond Thermogravimetric/Differential Thermal Analyzer, Perkin Elmer) and the Spectrum 100 Series (Perkin Elmer, Shelton, Connecticut, U S) equipment, with enhanced applications in various fields of activity such as the food, pharmaceutical, petrochemical sectors, respectively. This experimental investigation focused on revealing the compatibility between the active substance represented by theobromine and the mentioned sweeteners, under ambient conditions but also at high temperatures, showing that theobromine has a high thermic stability that leads to the idea that this active substance may be used at higher temperatures to obtain sweets that may be administered to children for the prevention of dental caries.

**The second study** is described in **the fifth chapter**, which explores the development and sensory evaluation of two novel proposed recipes of natural hot chocolate compared to a reference commercially available hot chocolate product. The study aimed to produce two hot chocolate formulations designed to be healthier, with lower sugar and higher diet fibre contents, but with sensory qualities comparable to traditional products. In this comparative study, the two hot chocolate recipes were subjected to sensory evaluations to determine their basic characteristics, especially flavour, texture, smell and consistency, to obtain a final well-structured product with multiple benefits for general health. Both recipes were evaluated to determine the sensory differences and the acceptability among the participating subjects, to identify their preferences and to optimize the characteristics of the final products. The study included 54 subjects from the Faculty of Dental Medicine in Timișoara who participated in the sensory evaluation. The evaluation focused on five attributes: aspect, consistency, flavour, taste and smell. The research method was conducted using sensory analysis, the subjects were investigated by a questionnaire, and the results were statistically processed by the Mann-Whitney and Wilcoxon Sign Rank tests. The study demonstrated that the P1 recipe is superior in terms of sensory qualities and has a favourable nutritional profile, which makes it an attractive choice for those who are searching for a healthier option of hot chocolate. This study significantly contributes to functional foods, emphasizing the importance of developing products that are not only tasty but also beneficial for health. The vegan sugar-free hot chocolate developed in this study may be integrated into a healthy diet and may serve as a model for future innovations in the food industry.

**The third study** described in **the sixth chapter**, offers a new perspective for an optimized recipe regarding the sweetener: sorrel or birch sugar. Starting from this idea, a second sensory evaluation was conducted, again with a strong emphasis on the sensory characteristics that are so important for the long-term success of a new product. This sensory evaluation aims to optimise the hot chocolate product and identify the variant that optimally combines the taste, flavour and creamy texture respecting the natural preparation principles. The study takes place in multiple stages and uses rigorous sensorial evaluation methods to determine the superiority of the new product over the commercially available one. The recipes were prepared with attention to detail, the ingredients being finely ground and strictly weighed before being mixed with water at 70-80°C. The sensorial evaluation took place at the Faculty of Dental Medicine of the „Victor Babeş” University of Medicine and Pharmacy Timișoara, after obtaining the approval of the Commission for Ethics in Scientific Research. The subjects were recruited from the faculty and were instructed to evaluate the samples based on a questionnaire that included five characteristics: aspect/colour, consistency, flavour, taste and smell. The study concluded that product P1, sweetened with sorrel, was superior to the other two tested products in terms of flavour, taste and smell. The commercially available product only excelled in terms of consistency.

The study conducted in this chapter suggests that the new optimized recipe not only fulfils but also exceeds the qualities of the reference product in certain aspects, offering a healthier and more attractive option for consumers.

**The fourth study** described in **the seventh chapter** has the essential objectives to achieve a healthy hot chocolate improved with

plants rich in polyphenols and sweetened with sorrel leaves powder; the analysis of the nutritional composition of recipes, the antioxidant activity and the total and individual content of polyphenols of the final products; the evaluation of the antimicrobial activity against bacterial strains specific for dental caries and the determination of the in vitro toxicological profile to achieve the safety for human consumption but also to investigate consumers' acceptability. By reaching these objectives, the study wishes to develop and evaluate two innovative sugar-free, vegan hot chocolate products, with strictly natural ingredients, emphasizing their nutritional, antioxidant, antimicrobial and anti-cariogenic properties. The study includes a detailed methodology based on revealing the total polyphenols content, using the Folin-Ciocalteu method, which measured the total amount of polyphenols, considered essential compounds due to their antioxidant properties; the identification and quantification of individual polyphenols by liquid mass chromatography (LC-MS), method that allowed a detailed characterization of the polyphenolic composition of chocolate; the antioxidant capacity was evaluated by the CUPRAC method. The antimicrobial activity of chocolate was tested against bacterial strains involved in the development of dental caries, such as *Streptococcus mutans* and *Streptococcus sanguinis*. Researchers attributed the anti-cariogenic activity of chocolate to its high polyphenol content, having strong antibacterial properties. The nutritional profile of hot chocolate recipes reflects that both had similar energetic values of about 408 kcal/100g, which makes them comparable with traditional chocolate but with no added sugar. This study significantly contributes to the knowledge of functional foods, revealing the importance of developing products that are not only tasty but also beneficial for health. The sugar-

free vegan chocolate developed in this study may be integrated into a healthy diet and may serve as a model for future innovations in the food industry.