Medicine in Evolution | Volume XXXII | No. 2/2026

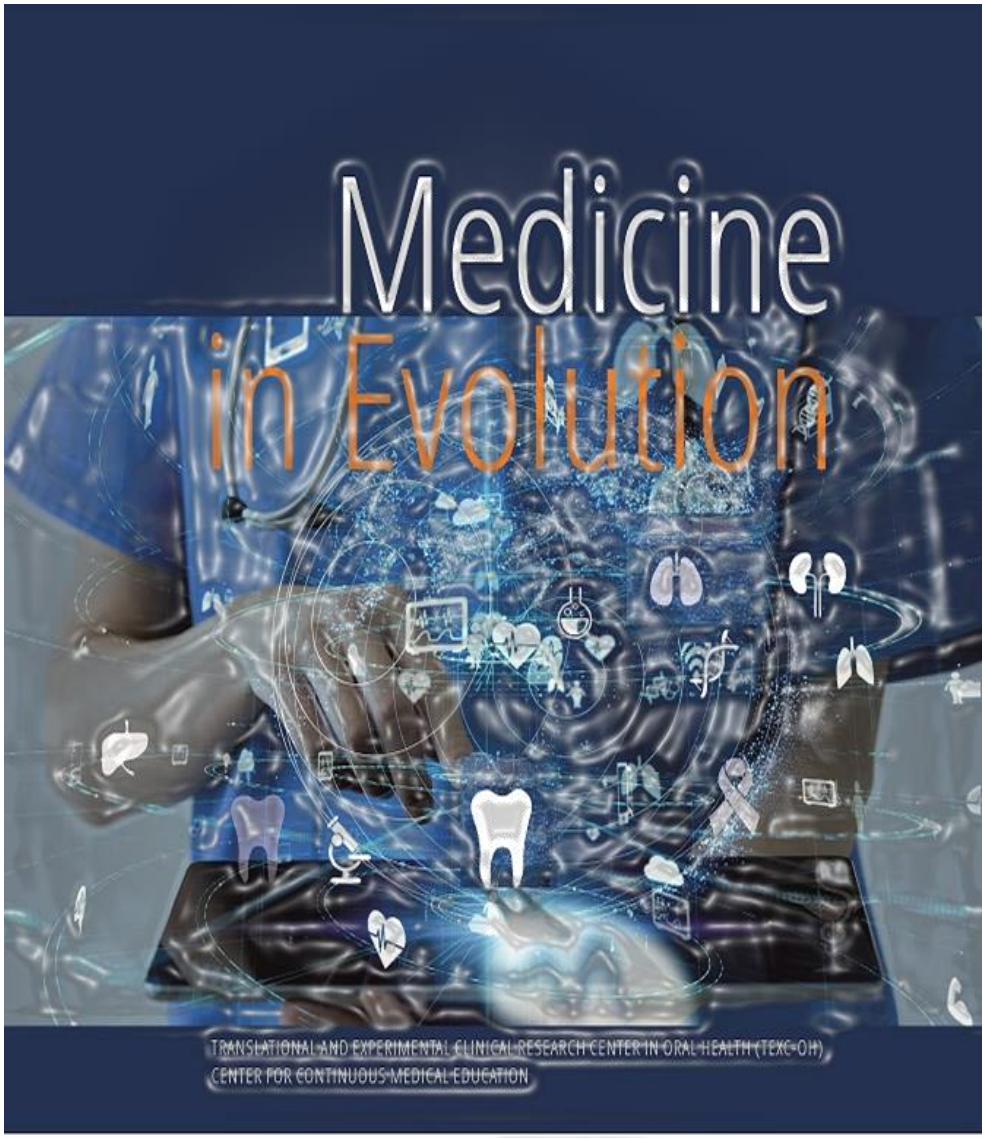
Medicine in Evolution

TRANSLATIONAL AND EXPERIMENTAL CLINICAL RESEARCH CENTER IN ORAL HEALTH (TEXC-OH)
CENTER FOR CONTINUOUS MEDICAL EDUCATION

ISSN 2065-376X medinevolution.umft.ro

Volume XXXII, No. 2, 2026, Timişoara, Romania
ISSN 2065-376X

MEDICINE IN EVOLUTION



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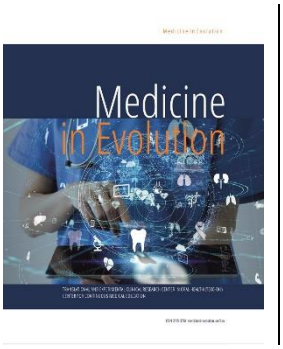

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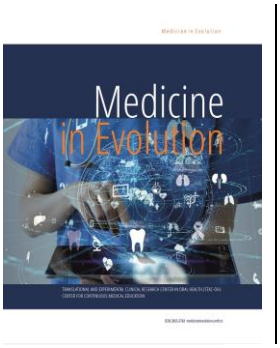
Background



The current journal was established by Prof. Dr. Mircea Ancusa in 1999, with the aim of acquiring knowledge and sharing insights in the noble profession guided by the principle "primum non nocere" (first, do no harm). In 2005, it was entrusted to a group of dedicated researchers at the Center of Health Education and Motivation for Prevention in Dentistry, under the leadership of Prof. Angela Codruta Podariu, DMD, PhD, at the Department of Preventive Dentistry of the University of Medicine and Pharmacy "Victor Babes" in Timisoara, Romania.

The inception of the journal stemmed from a dedication to exchange experiences in both professional and research domains. It was envisioned to encompass all medical specialties, with the aspiration that the published manuscripts would exhibit exceptional quality, elevating the journal's reputation. Esteemed professionals were enlisted to the editorial board and the review committee, individuals recognized for their expertise in the realm of research. The decision to publish papers in English was made to broaden accessibility to the global research community and enhance international recognition.

Since then, the journal has been regularly published under the auspices of the Center of Health Education and Motivation for Prevention in Dentistry, disseminating national and international research studies with the objective of evolving into a comprehensive evidence-based publication. Presently, the journal has transitioned to the stewardship of the Translational and Experimental Clinical Research Centre in Oral Health, situated within the Department of Preventive, Community Dentistry, and Oral Health. Its objectives are aligned with the vision of esteemed organizations such as the World Health Organization and the International Dental Federation, seamlessly integrating into the research strategy of Victor Babes University of Medicine and Pharmacy Timisoara.

"Medicine in Evolution" stands as a distinguished, peer-reviewed, open access journal dedicated to the dissemination of original theoretical research spanning the interdisciplinary spectrum of medicine and healthcare. Encompassing various topics within the realms of human life sciences, medical community, dental medicine, and pharmacology, the journal warmly welcomes original research papers, communications, letters, short notes, case reports, and reviews for submission. Committed to conducting rigorous peer reviews and expediting the publication of groundbreaking research, its mission is to advance the field of medicine through scholarly discourse.

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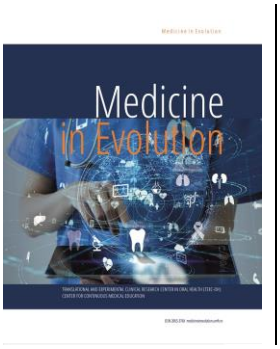

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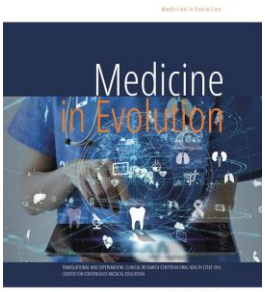

https://doi.org/10.70921/medev.v32i2.2032

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Received: 6 April 2026; Accepted: 4 May 2026; Published: 30 June 2026

Abstract

1.Background/Objectives: The integration of intraoral scanning, CAD/CAM technologies, and additive manufacturing has enabled the development of fully digital workflows in prosthodontics; however, their application in chairside anterior rehabilitations remains less documented. This clinical case aimed to evaluate a complete digital, chairside protocol for anterior maxillary rehabilitation, emphasizing digital planning and staged provisionalization. 2. Case Presentation: A 50-year-old male patient with partial anterior maxillary edentulism was treated using a fully digital workflow, including intraoral and facial scanning, Digital Smile Design, CAD-based wax-up, and fabrication of 3D-printed eggshell and on-abutment provisional restorations, followed by chairside milling of monolithic zirconia fixed prostheses. 3.Results: The staged provisional approach enabled validation of aesthetics and function, guided minimally invasive tooth preparation, and facilitated soft tissue conditioning. The final restorations demonstrated satisfactory marginal adaptation, occlusion, and aesthetic integration, with high patient acceptance. 4.Conclusion: A fully digital, chairside workflow allowed predictable and efficient anterior rehabilitation, improving treatment control, communication, and patient involvement, supporting its clinical applicability in modern prosthodontic practice within the limitations of a single case.

Keywords: Digital dentistry; intraoral scanning; CAD/CAM; chairside workflow; anterior prosthetic rehabilitation; Digital Smile Design; 3D printing; provisional restorations; ovate pontic; monolithic zirconia.

INTRODUCTION

The integration of digital technologies into prosthodontics has fundamentally transformed the diagnosis, planning, and execution of restorative treatments. In particular, the development of intraoral scanners, computer-aided design/computer-aided manufacturing (CAD/CAM) systems, and additive manufacturing has enabled the implementation of fully digital workflows, offering new possibilities for precision, efficiency, and patient-centered care [1].

Intraoral scanning represents a key component of this transformation, providing accurate digital impressions while eliminating many of the limitations associated with conventional impression techniques. Clinical studies and systematic reviews have demonstrated that intraoral scanners can achieve comparable or superior accuracy in fixed prosthodontics, while also improving patient comfort and reducing clinical time [2,3]. These advantages are especially relevant in anterior rehabilitations, where high esthetic demands require precise data acquisition and reproducibility.

Beyond data acquisition, the integration of digital tools into treatment planning has enabled the concept of facially driven prosthetic design, where dental restorations are planned in harmony with facial features and smile dynamics. Digital Smile Design (DSD) has emerged as a valuable tool for improving communication between clinician, technician, and patient, as well as for enhancing predictability by allowing visualization of the final outcome prior to treatment initiation [4].

The evolution toward a fully digital chairside workflow from intraoral scanning to CAD design and CAM fabrication has further streamlined clinical and laboratory procedures. Such workflows can reduce manual errors, improve consistency between treatment phases, and allow rapid iteration of designs, particularly when combined with additive manufacturing technologies such as 3D printing [1,5]. The use of 3D-printed provisional restorations has gained increasing attention due to their cost-efficiency, reproducibility, and ability to accurately transfer digital designs into the clinical environment [6].

Provisional restorations play a critical role in prosthodontic treatment, particularly in the anterior region, where they serve not only as temporary restorations but also as diagnostic tools for evaluating esthetics, function, and soft tissue response. Concepts such as ovate pontic design and staged provisionalization have been shown to support gingival contouring and emergence profile development, contributing to improved esthetic outcomes [7]. When integrated within a digital workflow, provisional restorations can be precisely designed and modified, allowing progressive validation of the treatment plan before fabrication of definitive restorations.

Additionally, the development of chairside CAD/CAM systems has enabled clinicians to perform multiple steps of the restorative process within a single clinical setting. Chairside workflows offer advantages such as reduced treatment time, elimination of conventional laboratory steps, and increased control over the entire process, although they may be limited by equipment capabilities and material constraints [8].

Despite these advancements, the application of fully digital, chairside workflows in multi-unit, tooth-supported anterior rehabilitations remain less documented compared to single-unit or implant-supported restorations. Therefore, further clinical reports are necessary to evaluate their feasibility, advantages, and limitations.

Aim and objectives

The aim of this clinical case report is to describe a complete digital chairside workflow for anterior maxillary prosthetic rehabilitation, highlighting the role of intraoral scanning,

facially driven design, staged provisionalization, and CAD/CAM fabrication in achieving a predictable functional and esthetic outcome.

CASE DESCRIPTION

Ethics, Case Introduction, and Clinical Objectives

This clinical case was approved by the Research Ethics Committee of the Victor Babeş University of Medicine and Pharmacy (Approval No. 62/06.11.2025) and was conducted in accordance with the principles outlined in the Declaration of Helsinki. The patient provided written informed consent for participation, including permission for the use of clinical data and photo-video documentation for scientific purposes.

A 50-year-old male patient presented to the Department of Prosthodontics, Faculty of Dental Medicine, seeking prosthetic rehabilitation for missing maxillary right canine and maxillary left lateral incisor (teeth 1.3 and 2.2). The patient was informed about all available treatment options, including implant-supported prosthetic rehabilitation; however, he declined implant therapy and opted for a fixed dental prosthetic solution supported by natural abutment teeth.

Clinical and digital evaluation protocols were implemented, including intraoral examination, photographic documentation, intraoral and facial scanning, and radiographic assessment. Based on these findings, a fully digital chairside workflow was selected.

Initial Clinical Examination and Digital Diagnostic Protocol

A comprehensive intraoral examination was performed, revealing no clinical signs of periodontal disease. The periodontal tissues were clinically healthy, with no evidence of gingival inflammation, bleeding on probing, attachment loss, or pathological tooth mobility. The oral hygiene status was satisfactory, and the remaining dentition exhibited stable periodontal support, making it suitable for prosthetic rehabilitation using natural abutments.

Occlusal analysis identified the presence of premature contacts and minor occlusal interferences, likely associated with the loss of dental units and compensatory tooth wear. These discrepancies were managed through selective enamel recontouring, aiming to establish a stable and functional occlusal scheme prior to initiating the digital diagnostic workflow. This preliminary equilibration step was essential to ensure an accurate and reproducible maxillomandibular relationship during intraoral scanning and digital bite registration.

An evaluation of the vertical dimension of occlusion (VDO) was also conducted through clinical assessment of facial proportions, interocclusal rest space, phonetics, and occlusal relationships. The analysis indicated that the VDO was within physiological limits and did not require modification. The stomatognathic system demonstrated adequate adaptive capacity, compensating for the existing tooth wear and partial edentulism without functional or aesthetic compromise. Therefore, the treatment plan was developed without altering the vertical dimension.

A comprehensive digital diagnostic protocol was subsequently implemented to assess the initial clinical condition and facilitate prosthetic treatment planning. This protocol included standardized intraoral and extraoral photographic documentation, intraoral scanning for digital impression acquisition, bite registration, and panoramic radiographic evaluation. These diagnostic tools allowed for a detailed assessment of both hard and soft tissues, as well as occlusal relationships, forming the foundation for a fully digital workflow.

Extraoral photographs were captured using a DSLR camera (Nikon D7500, Nikon Corp., Tokyo, Japan) equipped with an AF-S Micro Nikkor 85 mm lens and a Fixlite Twin Softbox flash system (Figure 1). Intraoral photographs were obtained using the same photographic setup to ensure consistency and high image quality (Figure 1).

Figure 1. Extraoral and intraoral photographs of the patient

Digital impressions of both arches, along with the interocclusal record, were acquired using an intraoral scanner (Medit i700, Medit Corp., Seoul, South Korea). The use of intraoral scanning enabled precise capture of dental and gingival structures while improving patient comfort and eliminating the need for conventional impression materials.

A facial scan was subsequently performed using the face-scanning function of the same intraoral scanner (Medit i700, Medit Corp., Seoul, South Korea), capturing key facial landmarks including the lips, nose, and surrounding anatomical structures. The intraoral scan data were then imported and aligned with the facial scan mesh within the Medit Link software (Medit Corp., Seoul, South Korea), allowing accurate integration of dental and facial datasets for prosthetically driven treatment planning.

All acquired digital data were exported and imported into a dental CAD software (DentalCAD, exocad GmbH, Darmstadt, Germany) for analysis, planning, and design. A Digital Smile Design (DSD) was developed using the Smile Creator module, which utilized facial reference lines and proportions to guide the aesthetic design of the future restorations in harmony with the patient's facial features.

Following a comprehensive assessment of the clinical findings and a detailed discussion of available treatment options with the patient, a fully digital workflow was selected. The treatment plan included the fabrication of custom "eggshell" 3D-printed interim restorations, indirect on-abutment 3D-printed provisional restorations, and definitive full-contour monolithic zirconia fixed partial dentures (FPDs) (IPS e.max ZirCAD, Ivoclar, Schaan, Liechtenstein).

Digital Wax-Up, 3D Printing, and Fabrication of Provisional Restorations

Based on the diagnostic data and the Digital Smile Design (DSD), a virtual diagnostic wax-up was created using dental CAD software (DentalCAD, exocad GmbH, Darmstadt, Germany), serving as the foundation for the prosthetically driven treatment plan (Figure 2). This digital wax-up allowed visualization of the anticipated functional and aesthetic outcome and facilitated the subsequent fabrication of both provisional and definitive restorations.

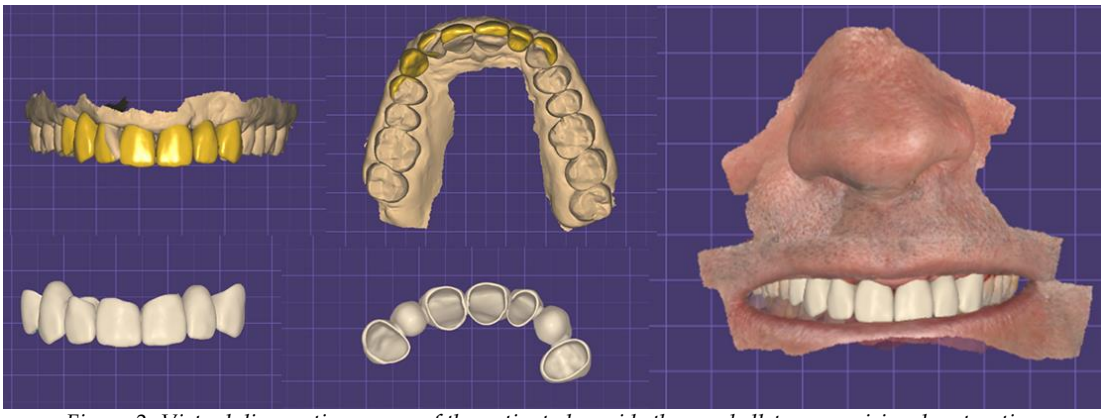

Figure 2. Virtual diagnostic wax-up of the patient alongside the eggshell-type provisional restoration

The virtual design was exported and processed for additive manufacturing. A physical model of the wax-up was fabricated using a stereolithographic 3D printer (Anycubic Photon M5s, Anycubic, Shenzhen, China) and a high-speed gray model resin, previously calibrated according to the manufacturer's specifications to ensure dimensional accuracy and optimal surface quality.

An eggshell-type provisional restoration was then digitally designed based on the validated wax-up. This restoration was intended to function as an indirect interim prosthesis, to be relined intraorally following tooth preparation. The provisional restoration extended across teeth 1.4, 1.2, 1.1, 2.1, and 2.3, with teeth 1.3 and 2.2 replaced as pontics.

The provisional shells were fabricated using a stereolithographic 3D printer (Prusa SL1, Prusa Research, Prague, Czech Republic) and a ceramic-reinforced crown and bridge resin (SprintRay Crown, SprintRay Inc., Los Angeles, CA, USA). Printing parameters were adjusted in accordance with the manufacturer's recommended exposure settings to ensure optimal polymerization and mechanical properties.

A key design feature of the shell provisional restorations was the incorporation of an ovate pontic morphology for teeth 1.3 and 2.2. This design was intentionally selected to promote soft tissue contouring and support the development of a natural emergence profile in the edentulous areas. The ovate pontic exerted controlled pressure on the healing mucosa approximately two months post-extraction, facilitating the shaping of the gingival architecture without the need for surgical intervention. This approach was particularly relevant given that the patient declined all surgical procedures, including gingivectomy, alveolar ridge modification, or other soft tissue recontouring techniques.

Although gingival recontouring was initially considered as part of the treatment plan to optimize the gingival aesthetic line (GAL), it was ultimately excluded due to the patient's explicit refusal. Clinical evaluation of the smile line revealed minimal gingival exposure, which, in combination with the patient's preferences, allowed continuation of the treatment without surgical modification of the gingival margins, accepting a compromise in achieving an idealized gingival aesthetic line.

To transfer the digital wax-up into the clinical setting, a silicone index was fabricated using a condensation silicone material (Zetaplus System, Zhermack SpA, Badia Polesine, Italy). The index was then loaded with a bis-acrylic crown and bridge material (LuxaCrown, DMG, Hamburg, Germany) and used to fabricate a direct intraoral mock-up.

This mock-up served both as a functional and aesthetic preview of the proposed treatment outcome (Figure 3) and as a clinical guide for minimally invasive, prosthetically driven tooth preparation. It enabled precise control over reduction depth while ensuring preservation of tooth structure and alignment with the final restorative design.

Figure 3. Facial and intraoral view of the direct intraoral mock-up

Tooth Preparation, Gingival Retraction, and Digital Impressions

All clinical procedures were performed under local anesthesia. The abutment teeth were prepared for full-coverage zirconia restorations using a minimally invasive, prosthetically driven approach guided by the previously validated digital wax-up and intraoral mock-up. Tooth preparation was carried out with the aim of preserving maximum dental structure while ensuring adequate space for the restorative material and optimal marginal adaptation.

Gingival displacement was achieved using a single-cord retraction technique. Considering the patient's thin gingival biotype, a size 00 retraction cord (Ultrapak, Ultradent Products Inc., South Jordan, UT, USA) was selected. The cord was carefully and atraumatically placed circumferentially into the sulcus, slightly apical to the preparation margins, to allow proper exposure of the finish lines while minimizing the risk of soft tissue trauma. This approach ensured optimal visualization and accessibility for accurate impression capture.

Following gingival retraction, digital impressions of the prepared teeth were obtained using the same intraoral scanner (Medit i700, Medit Corp., Seoul, South Korea), previously calibrated according to the manufacturer's instructions. High-resolution full-arch scans of both the maxillary and mandibular arches were acquired, along with an interocclusal record, to accurately capture occlusal relationships and spatial positioning. The use of intraoral scanning allowed for immediate verification of scan quality and margin clarity, contributing to the precision of the subsequent digital workflow.

Subsequently, the previously fabricated 3D-printed eggshell provisional restorations were relined chairside using a bis-acrylic crown and bridge material (LuxaCrown, DMG, Hamburg, Germany). This relining procedure ensured optimal marginal adaptation and internal fit of the provisional restorations to the prepared abutment teeth.

The provisional restorations were then cemented using a eugenol-free temporary cement (TempBond NE, Kerr Corporation, Orange, CA, USA), allowing for adequate retention while avoiding interference with the polymerization of future resin-based luting agents.

In addition to providing immediate functional and aesthetic rehabilitation, the provisional restorations played a critical role in conditioning the soft tissues. Specifically, the ovate pontic design exerted controlled pressure on the edentulous ridges, promoting gingival contouring and the development of a favorable emergence profile in the pontic areas. This

phase was essential for achieving an optimal soft tissue architecture prior to the fabrication of the definitive prosthetic restorations.

Second Provisionalization and Final Digital Impression

After one week of healing with the initial eggshell provisional restorations in place, a second set of provisional restorations was fabricated and prepared for clinical insertion. This intermediate phase aimed to further refine gingival contouring and to clinically validate the definitive prosthetic design in terms of aesthetics, function, and patient acceptance.

The second set of provisionals was designed based on the previously acquired intraoral scan obtained after tooth preparation, which accurately reflected the prepared abutments and the post-preparation clinical situation. Unlike the initial shells, these restorations were conceived as full-contour, on-abutment provisional restorations, closely replicating the morphology and aesthetic parameters of the intended definitive prostheses.

The provisional restorations were fabricated using a stereolithographic 3D printer (Prusa SL1, Prusa Research, Prague, Czech Republic) and a ceramic-reinforced crown and bridge resin (SprintRay Crown, SprintRay Inc., Los Angeles, CA, USA). This material selection allowed for improved mechanical resistance and surface quality, making it suitable for functional provisionalization in the anterior region.

Upon removal of the initial eggshell provisionals, a favorable soft tissue response was observed, with evident gingival contouring and maturation in the edentulous areas, particularly at the sites corresponding to teeth 1.3 and 2.2 (Figure 4). The ovate pontic design of the initial provisionals had successfully contributed to the shaping of the peri-prosthetic soft tissues, resulting in a more natural emergence profile.

Figure 4. Favorable emergence profile observed in the pontic areas (teeth 1.3 and 2.2) after 7 days of provisionalization. The ovate pontic design exerted controlled pressure on the edentulous ridges, contributing to soft tissue contouring and the development of a natural gingival architecture

The second set of provisional restorations was carefully adjusted for marginal fit, proximal contacts, and occlusion, and subsequently cemented using a eugenol-free temporary cement (TempBond NE, Kerr Corporation, Orange, CA, USA). These restorations served as a functional and aesthetic prototype of the final prosthetic outcome.

The patient expressed full satisfaction with the aesthetic appearance and overall design of this provisional stage, effectively approving the definitive restorative concept.

Following an additional one-week period to allow for further soft tissue stabilization and maturation, a final intraoral digital impression was obtained.

This definitive digital impression captured the fully conditioned gingival architecture and the stabilized prosthetic field, providing the basis for the fabrication of the final restorations. The previously validated digital design was then adapted to this final clinical situation, ensuring optimal integration of functional, biological, and aesthetic parameters in the definitive prosthetic outcome.

Laboratory Workflow and Fabrication of Final Restorations

All digital records including the final intraoral impressions, preoperative photographic documentation, Digital Smile Design (DSD), facial scan, and validated digital wax-up were imported into dental CAD software (DentalCAD, exocad GmbH, Darmstadt, Germany). These datasets were used to refine and adapt the previously established prosthetic design to the final clinical situation, ensuring consistency between the provisional phase and the definitive restorations.

Based on the validated esthetic and functional parameters, the definitive restorations were digitally designed as full-contour monolithic zirconia prostheses (Figure 5). To maintain a fully chairside workflow and accommodate the technical limitations of the milling unit, the restorations were strategically divided into three separate components. This approach was dictated by the maximum milling capacity of the chairside system, which allowed fabrication of up to three-unit fixed partial dentures. Accordingly, the definitive restorations were segmented as follows: one full-contour monolithic zirconia fixed partial denture (FPD) replacing tooth 1.3, supported by teeth 1.4 and 1.2; one single full-contour monolithic zirconia crown on tooth 1.1; one full-contour monolithic zirconia FPD replacing tooth 2.2, supported by teeth 2.1 and 2.3. This segmentation enabled complete chairside fabrication without compromising the prosthetic design or clinical outcome.

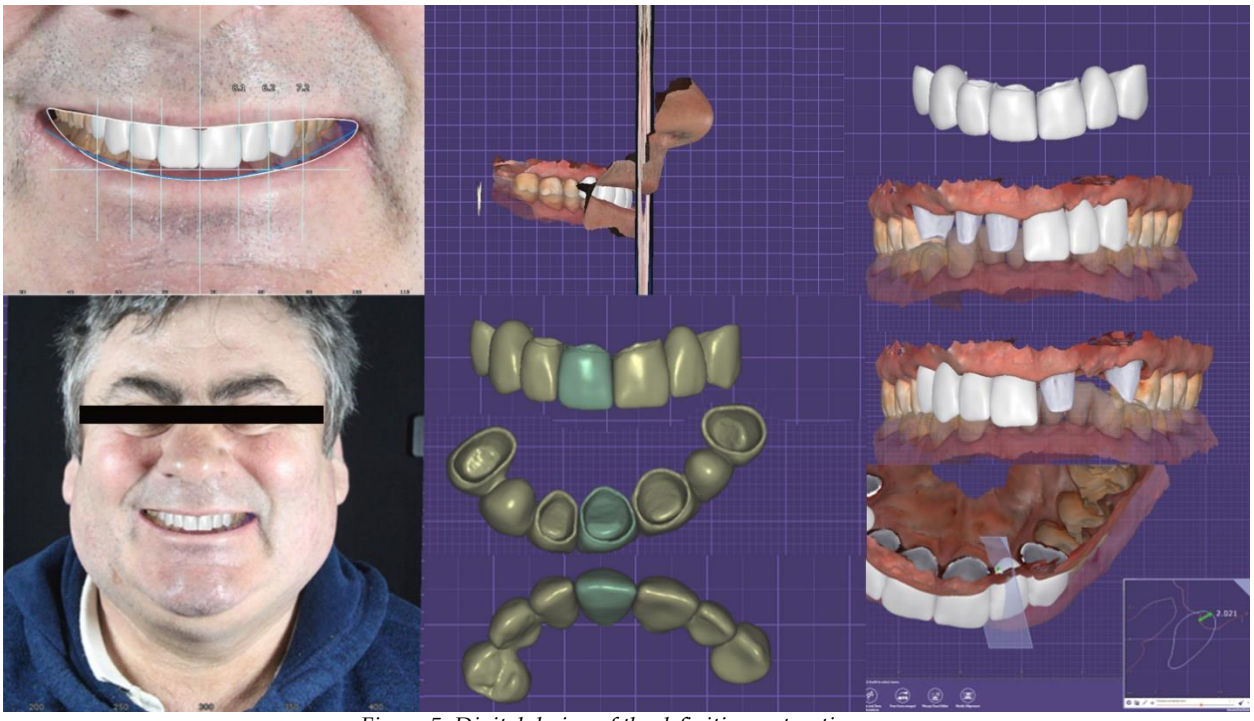

Figure 5. Digital design of the definitive restorations

The restorations were milled from zirconia blocks (IPS e.max ZirCAD HT/B45, Ivoclar, Schaan, Liechtenstein), a material characterized by high flexural strength and favorable optical properties suitable for anterior restorations. The milling procedure was

performed using a chairside milling unit (Planmeca PlanMill 40, Planmeca Oy, Helsinki, Finland).

Following milling, the restorations were finished and polished in accordance with the manufacturer's recommendations and subsequently sintered in a high-temperature furnace (Programat CS6, Ivoclar, Schaan, Liechtenstein) to achieve their final mechanical properties and translucency.

Characterization was performed through selective staining of the buccal surfaces to enhance aesthetic integration. The restorations were polished rather than glazed, particularly on the occlusal surfaces, in order to preserve the precise occlusal morphology and contact relationships established during the digital design phase.

The completed restorations were then subjected to a clinical try-in, during which marginal adaptation, proximal contacts, occlusion, and overall aesthetics were carefully evaluated. Both the clinician and the patient approved the final outcome.

Definitive cementation was performed using a resin-modified glass ionomer cement (GC FujiCEM 2, GC Corporation, Tokyo, Japan), ensuring adequate retention and marginal seal. The final cemented restorations are presented in Figure 6 alongside the overall aesthetic outcome.

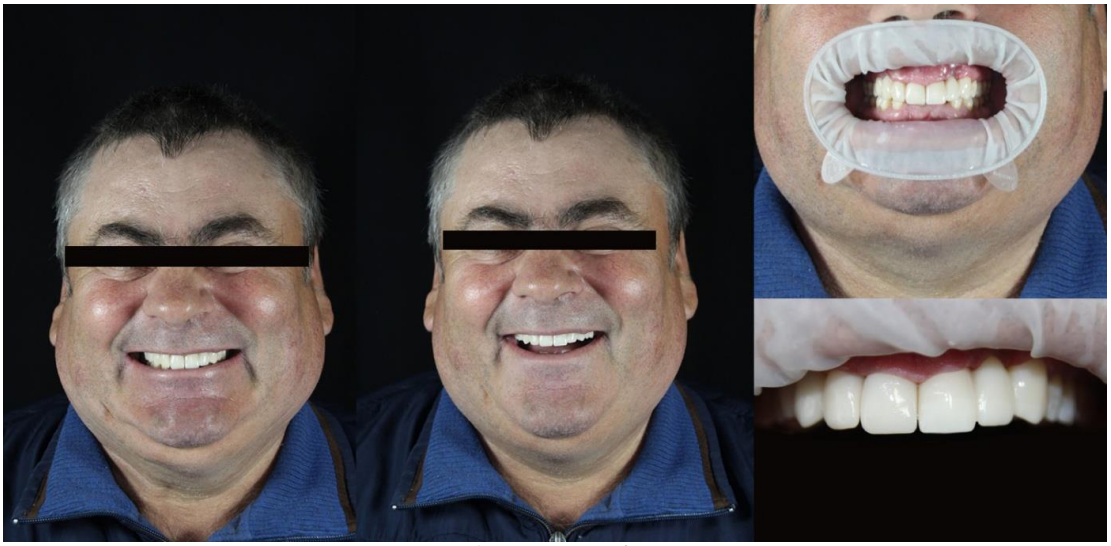

Figure 6. Final restorations after cementation

DISCUSSIONS

This clinical case illustrates how a fully digital chairside workflow, centered on intraoral scanning and chairside CAD/CAM technologies, can be used to rehabilitate the anterior maxilla in a patient who declined implant and surgical soft-tissue procedures. One of the principal strengths of the protocol was the integration of diagnosis, simulation, provisionalization, and definitive restoration into a coherent digital sequence. Current evidence supports the growing role of fully digital chairside workflows in fixed prosthodontics, particularly because they may improve time efficiency, streamline communication between clinical and technical phases, and enhance patient-centered outcomes. However, the literature also emphasizes that evidence remains limited for complex tooth-supported restorations, and further clinical validation is required [1].

A major strength of the present treatment was the use of intraoral scanning as the foundation for all subsequent steps. Intraoral scanners have significantly improved impression accuracy, workflow efficiency, and patient comfort compared with conventional

impression techniques. A systematic review demonstrated that digital impressions are generally preferred by patients and may reduce clinical working time while maintaining comparable accuracy [2]. These advantages are particularly relevant in anterior rehabilitations, where precision and repeatability are critical.

The fully digital chairside workflow enhanced predictability by integrating all diagnostic and therapeutic data photographs, intraoral scans, facial scans, Digital Smile Design, and CAD planning into a unified workflow. Systematic reviews have reported that fully digital chairside workflows can be efficient and clinically acceptable; however, they also highlight variability in outcomes and the need for more standardized long-term studies [1].

Another important strength was the use of facially driven planning through Digital Smile Design (DSD). This approach allows clinicians to integrate facial proportions, smile dynamics, and dental morphology into treatment planning. Recent studies suggest that DSD improves communication with patients and enhances treatment acceptance by enabling visualization of the final outcome before intervention [4,9,10].

The use of a diagnostic wax-up and mock-up-guided preparation contributed significantly to the minimally invasive nature of the treatment. The literature supports that prosthetically driven preparation based on a mock-up reduces unnecessary tooth reduction and allows more controlled and conservative preparation, especially in esthetically demanding cases [10,11].

A distinctive aspect of this case was the use of an initial 3D-printed eggshell provisional restoration with ovate pontics. This approach provided several advantages: it transferred the digital wax-up accurately to the oral environment, reduced chairside time, and facilitated non-surgical soft-tissue conditioning. Studies have shown that ovate pontic designs can improve gingival contour and emergence profile, particularly in post-extraction sites, enhancing esthetic outcomes in the anterior region [12].

The staged use of multiple provisional restorations further increased treatment predictability. The first provisional facilitated gingival conditioning, while the second allowed functional and esthetic validation of the final design. This stepwise approach aligns with prosthodontic principles that emphasize provisional restorations as diagnostic tools rather than merely temporary solutions [11].

The use of 3D printing for provisional restorations offered advantages in cost-efficiency, reproducibility, and speed. However, systematic reviews indicate that while 3D-printed provisional materials show clinically acceptable marginal accuracy, they may exhibit inferior mechanical properties compared to milled alternatives, depending on the material and processing protocol [6].

The chairside workflow represented another major strength. It allowed for complete control of all clinical and technical steps, reduced treatment time, and minimized dependency on external laboratory processes. Chairside CAD/CAM systems have been shown to provide efficient and esthetically satisfactory restorations with reduced turnaround time [8].

Nevertheless, the fully digital chairside workflow introduced limitations. The most significant constraint was the milling unit's limitation to a maximum of three-unit restorations, necessitating segmentation of the prosthetic design. While this did not compromise the outcome in the present case, it may represent a limitation in more extensive rehabilitations. Additionally, current evidence suggests that digital workflows are less validated for larger-span prostheses and more complex cases [13].

The selection of monolithic zirconia as the definitive restorative material provided high mechanical strength and acceptable esthetics. Clinical studies have demonstrated favorable performance of digitally fabricated zirconia restorations, although long-term data especially for multi-unit restorations remain limited [14].

A notable limitation of this case was the inability to surgically optimize the gingival aesthetic line (GAL) due to the patient's refusal of surgical intervention. However, given the minimal gingival display during smiling, the esthetic outcome remained acceptable. This highlights the importance of individualized, patient-centered treatment planning.

A critical limitation of this workflow relates to the constraints of chairside CAD/CAM systems. The necessity to segment the prosthetic design due to milling limitations may introduce additional interfaces, which could potentially affect long-term mechanical stability and prosthetic integrity. Furthermore, although 3D-printed provisional materials offer advantages in speed and reproducibility, their mechanical properties and wear resistance remain inferior to milled alternatives, which may limit their use in extended provisional phases. Another important consideration is the learning curve associated with fully digital chairside workflows, as well as the financial investment required for equipment and software integration. These factors may influence the broader clinical applicability of such protocols.

Finally, it must be acknowledged that this report represents a single clinical case, and therefore its findings cannot be generalized. Future research should focus on long-term clinical outcomes, comparison between digital and conventional workflows, and further evaluation of 3D-printed materials and chairside limitations.

CONCLUSIONS

This clinical case demonstrates that a fully digital, chairside workflow can provide a predictable and efficient approach for anterior prosthetic rehabilitation. The integration of intraoral scanning, facially driven digital planning, Digital Smile Design, and CAD/CAM technologies enabled precise control over diagnosis, treatment planning, and execution.

The use of staged provisional restorations including eggshell and on-abutment 3D-printed provisionals played a key role in validating aesthetics and function, improving patient communication, and allowing progressive soft tissue conditioning. Mock-up-guided preparation further supported a minimally invasive, prosthetically driven approach.

Within the limits of a single clinical case, this protocol highlights the clinical advantages of digital technologies, including improved workflow efficiency, enhanced predictability, and greater patient involvement, supporting their growing role in modern prosthodontic practice.

Author Contributions

Conceptualization, A.-B.F, A.J.; methodology, A.J., I.T., A.-E.A.-L., R.R. and A.-B.F.; software, A.-B.F., I.T., R.R. and A.-E.A.-L.; validation, A.J., I.T. and A.-B.F.; formal analysis, A.J. and A.-B.F.; investigation, A.-E.A.-L.; resources, A.J., A.-E.A.-L., I.T., R.R. and A.-B.F.; data curation, A.J., A.-B.F., I.T., R.R. and A.-E.A.-L.; writing, A.J., R.R. and A.-B.F.; writing-review and editing, A.J., R.R. and A.-B.F.; visualization, A.-E.A.-L., I.T., R.R. and A.-B.F.; super-vision, A.J., R.R. and A.-B.F.; project administration, A.J. All authors have read and agreed to the pub-lished version of the manuscript.

Institutional Review Board Statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of the Victor Babes University of Medicine and Pharmacy Timisoara (approval No.62/06.11.2025)

Informed Consent Statement

Informed consent was obtained from the subject involved in this study. Written informed consent has been obtained from the patient to publish this paper.

Data Availability Statement

The data presented in this study is available on request from the corresponding author.

Acknowledgments

The Article Processing Charge (APC) was funded by "Victor Babes" University of Medicine and Pharmacy in Timisoara, Romania.

Conflicts of Interest

The authors declare no conflict of interest.

The APC was funded by "Victor Babes" University of Medicine and Pharmacy in Timisoara, Romania.

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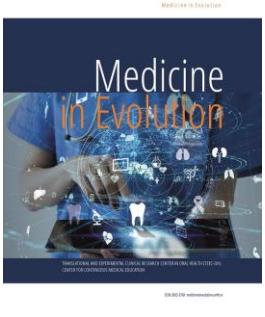
Hypoglycemic Bread as a Nutritional Strategy for the Management of Diabetes Mellitus



https://doi.org/10.70921/medev.v32i2.2035

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Received: 14 May 2026; Accepted: 26 May 2026; Published: 30 June 2026

Abstract

Diabetes mellitus represents a major global health challenge, requiring effective nutritional strategies to improve glycemic control and metabolic outcomes. The aim of this study was to evaluate the impact of bakery product reformulation on nutritional composition and sensory acceptability in the context of functional nutrition for patients with carbohydrate metabolism disorders. A comparative experimental design was applied to four bread formulations: white bread (control), rye bread, whole grain bread, and buckwheat bread with chia seeds. Nutritional composition was determined by indirect calculation methods, and sensory evaluation was performed using a hedonic scoring method with a semi-trained panel. The results showed a significant reduction in carbohydrate content from 41.13 g/100 g in white bread to 26.92 g/100 g in buckwheat bread with chia seeds ($p < 0.05$), along with a substantial increase in dietary fiber up to 10.10 g/100 g in rye bread. In addition, the energy value decreased from 247.09 kcal/100 g to 161.67 kcal/100 g. Sensory analysis indicated high acceptability for all formulations, with overall scores exceeding 4.0. In conclusion, the reformulation of bakery products using whole grains, pseudocereals, and functional ingredients represents an effective and practical strategy for improving dietary quality and supporting glycemic control in diabetes management without compromising consumer acceptability.

Keywords: diabetes mellitus; glycemic control; functional bakery products; dietary fiber; hypoglycemic bread; whole grains; pseudocereals; sensory analysis; clinical nutrition

INTRODUCTION

1.1 DIETARY MANAGEMENT IN DIABETES MELLITUS

Diabetes mellitus represents one of the most significant chronic metabolic diseases of the 21st century, characterized by persistent hyperglycemia resulting from defects in insulin secretion and/or action. This condition is associated with high morbidity and mortality rates, mainly due to its cardiovascular, neurological, and renal complications, constituting a major global public health challenge [1]. From an endocrinological perspective, diabetes involves a complex imbalance in energy homeostasis and carbohydrate metabolism, being closely associated with insulin resistance and pancreatic β-cell dysfunction.

In modern diabetes management, medical nutrition therapy plays a central role and is considered an essential and effective intervention for glycemic control and the reduction of complication risk [1]. Current evidence indicates that dietary interventions can achieve significant improvements in glycated hemoglobin (HbA1c), in some cases comparable to pharmacological treatments [2]. Therefore, optimizing dietary composition, particularly carbohydrate intake, represents a key strategy in the metabolic management of patients with diabetes mellitus.

Carbohydrates are the primary determinant of postprandial glycemic response, and their quality is considered more important than the total quantity consumed [3]. In this regard, current guidelines recommend the consumption of carbohydrates from whole-food sources, rich in dietary fiber and micronutrients, while limiting refined products such as white bread and other bakery items with a high glycemic index [3]. Products derived from refined cereals are rapidly digested, leading to sharp increases in blood glucose levels and an exaggerated insulin response, thereby promoting metabolic stress and disease progression [4].

Bakery products occupy a central place in human nutrition, representing a major source of energy and carbohydrates worldwide. However, frequent consumption of white bread has been associated with an increased risk of developing type 2 diabetes, mainly due to its high content of available carbohydrates and elevated glycemic index [4]. Epidemiological studies have shown that diets characterized by a high glycemic index and low dietary fiber intake are associated with a higher incidence of diabetes mellitus and metabolic syndrome [4].

In contrast, increased dietary fiber intake and consumption of whole grains are associated with significant metabolic benefits. Cohort studies have demonstrated a 20–30% reduction in diabetes risk among individuals with higher fiber intake, particularly when derived from whole grains [5]. Dietary fibers exert their effects through multiple physiological mechanisms, including slowing carbohydrate digestion and absorption, increasing intestinal viscosity, modulating incretin hormone secretion (GLP-1, PYY), and favorably influencing gut microbiota composition [6].

Recent meta-analyses confirm the beneficial effects of fiber supplementation on glycemic control, demonstrating significant reductions in HbA1c, fasting glucose levels, and insulin resistance in patients with type 2 diabetes [6]. Furthermore, diets rich in fiber and complex carbohydrates have been associated with improvements in lipid profile and a reduction in cardiovascular risk, which is particularly relevant given the frequent comorbidities associated with diabetes [7].

From a food technology perspective, reformulation of bakery products represents a key strategy for developing functional foods tailored to the needs of diabetic patients. The concept of "functional bread" involves the use of whole grain flours, pseudocereals (such as buckwheat), and fiber-rich ingredients (seeds, soluble fibers) to reduce glycemic index and improve the nutritional profile of the final product [8]. Experimental studies have shown that bread formulated with such ingredients leads to a significant reduction in postprandial

glycemia compared to white bread, an effect correlated with increased fiber, protein, and lipid content [8].

Moreover, the physical structure of food plays an important role in determining glycemic response. Flour particle size, degree of processing, and the composition of the food matrix influence carbohydrate bioavailability and digestion rate [9]. Products made from less processed whole grains or coarser flours result in a lower glycemic response, highlighting the importance of processing technology in metabolic control [9].

Despite these nutritional benefits, the sensory acceptability of hypoglycemic bakery products remains a major challenge in the food industry. Organoleptic characteristics such as texture, taste, and appearance significantly influence consumer compliance, including among patients with diabetes mellitus. Therefore, the development of bakery products that combine metabolic benefits with desirable sensory properties represents a priority for both nutritional research and the food industry.

In this interdisciplinary context, at the intersection of endocrinology, clinical nutrition, and food technology, the present study aims to comparatively evaluate the nutritional and sensory profiles of different types of hypoglycemic bread, in order to assess their relevance as functional dietary interventions in the management of diabetes mellitus.

1.2 Clinical and metabolic relevance of hypoglycemic bakery products

The reformulation of staple foods such as bread represents an effective nutritional strategy for the management of metabolic disorders, particularly diabetes mellitus. The physiological impact of bakery products extends beyond macronutrient composition and is strongly influenced by structural, functional, and metabolic interactions within the food matrix and the gastrointestinal environment [11].

Current evidence indicates that several interconnected mechanisms contribute to the metabolic effects of hypoglycemic bakery products, including structural modifications of starch, dietary fiber functionality, appetite regulation, and sensory acceptability. These mechanisms act synergistically to modulate postprandial glycemic response, insulin sensitivity, and overall metabolic homeostasis [1].

Figure 1 synthesizes the multifactorial interactions underlying the metabolic effects of hypoglycemic bakery products, highlighting the integration of structural, physiological, and behavioral determinants. At the structural level, modifications of starch architecture—such as increased resistant starch content and reduced enzymatic accessibility—play a central role in attenuating glycemic excursions. In parallel, the incorporation of soluble and insoluble dietary fibers contributes to delayed gastric emptying, modulation of carbohydrate digestion, and enhanced fermentation in the colon, leading to the production of short-chain fatty acids with recognized metabolic benefits [12,13].

Furthermore, satiety regulation emerges as a critical pathway linking food formulation to energy intake control. The stimulation of gut-derived hormones involved in appetite regulation (e.g., GLP-1, PYY) may reduce subsequent caloric intake and improve long-term metabolic outcomes. Sensory acceptability, although often underestimated, represents an essential determinant of adherence, as the success of any reformulated product depends on its integration into habitual dietary patterns without compromising palatability [12,13].

Additionally, the figure emphasizes the role of food-based strategies within the broader context of metabolic disease management. By simultaneously targeting multiple physiological pathways, hypoglycemic bakery products may contribute to improved glycemic variability, enhanced insulin sensitivity, and reduced cardiometabolic risk. These effects extend beyond acute postprandial responses and may support long-term metabolic stability when such products are consistently included in the diet [14-16].

In addition to their direct metabolic effects, hypoglycemic bakery products have important practical implications for dietary interventions at the population level. As bread

and similar products constitute staple foods in many dietary patterns, their reformulation offers a scalable and sustainable approach to improving nutritional quality without requiring major behavioral changes. This aspect is particularly relevant in the context of diabetes prevention programs, where long-term adherence to dietary recommendations often represents a major challenge [15,16].

Moreover, the integration of such products into daily nutrition aligns with current public health strategies aimed at reducing the burden of non-communicable diseases. By lowering the glycemic impact of widely consumed foods, these reformulated products may contribute to improved metabolic control not only in individuals with diagnosed diabetes, but also in those at risk of developing insulin resistance and metabolic syndrome [15,16].

Future research should focus on optimizing formulation strategies through the combined use of novel ingredients, processing techniques, and personalization approaches. Advances in food technology, together with insights from metabolomics and gut microbiota research, may further enhance the efficacy of hypoglycemic bakery products. In this context, the development of tailored functional foods adapted to individual metabolic profiles represents a promising direction within the field of precision nutrition [13].

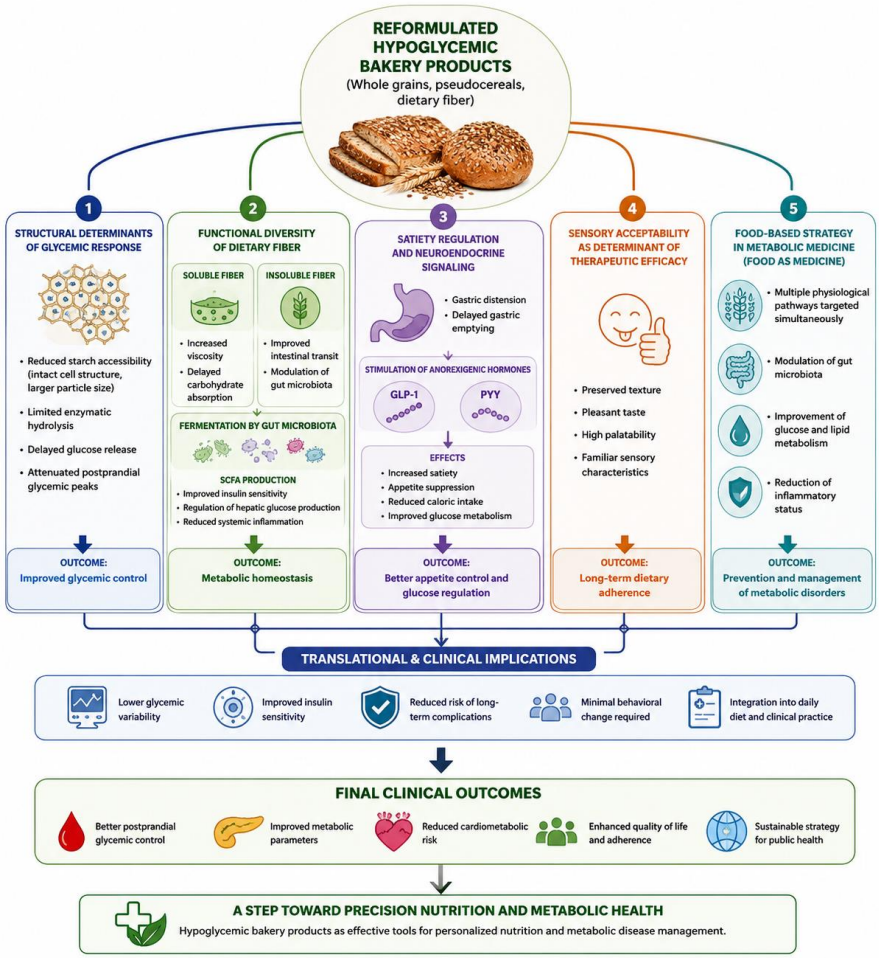

Figure 1. Conceptual framework of the clinical and metabolic effects of hypoglycemic bakery products (Figure created with ChatGPT-5 , OpenAI, San Francisco, CA, USA, accessed on 1 May 2026)

As illustrated in Figure 1, the metabolic impact of hypoglycemic bakery products arises from the interaction of multiple complementary mechanisms rather than a single isolated effect. Structural modifications that reduce starch accessibility contribute to slower

digestion kinetics and attenuated postprandial glycemic responses [11]. In parallel, dietary fiber plays a central role in modulating gastrointestinal function and metabolic processes, including gut microbiota activity and short-chain fatty acid (SCFA) production [12,13].

The figure also highlights the importance of satiety-related and behavioral components. The stimulation of gut-derived hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) supports appetite regulation and long-term energy balance, while sensory acceptability ensures adherence to dietary interventions [14,16].

Taken together, these interconnected mechanisms reinforce the concept of "food as medicine," emphasizing the potential of reformulated bakery products as practical and effective tools in the dietary management and prevention of metabolic disorders [15].

Aim and objectives

The present study was designed as a comparative experimental investigation aimed at evaluating the applicability of reformulated bakery products within the framework of functional nutrition for patients with carbohydrate metabolism disorders. The methodological approach was based on the controlled analysis of how raw material composition influences the final nutritional characteristics of the products, with particular emphasis on parameters relevant to the modulation of glycemic response.

The study design enabled a direct comparison between a conventional product, represented by white bread obtained from refined flour, and reformulated variants developed using alternative raw materials with enhanced nutritional value. Specifically, formulations based on rye flour, whole wheat flour, and buckwheat flour supplemented with chia seeds were selected, considering their distinct differences in dietary fiber content, carbohydrate structure, and nutrient bioavailability.

The experimental setup ensured strict standardization of technological parameters and processing conditions, allowing observed variations to be attributed exclusively to differences in raw material composition. Such standardization is essential, as the nutritional and metabolic properties of bakery products are influenced not only by their chemical composition but also by the interactions between macronutrients and the structure of the food matrix, which ultimately determine digestibility and postprandial glycemic response [8,9].

In addition, the study included an evaluation of the sensory characteristics of the developed products in order to determine their acceptability. This aspect is particularly important in a clinical context, as adherence to dietary interventions is strongly influenced by the sensory properties of foods. Reformulation strategies, especially those involving increased fiber content and the incorporation of pseudocereals, may affect texture, taste, and overall acceptability, thus requiring careful optimization to balance nutritional benefits with consumer acceptance.

Therefore, the main objective of the study was to comparatively evaluate the nutritional profile and sensory characteristics of reformulated bakery products, with the aim of identifying technologically and metabolically optimized formulations. At the same time, the study aimed to highlight the potential of these products to be integrated into modern clinical nutrition strategies, contributing to the development of effective and sustainable dietary interventions for the management of carbohydrate metabolism disorders.

MATERIAL AND METHODS

2.1 Raw and auxiliary materials used

The selection of raw materials used in the present study was carried out in accordance with the objective of developing bakery products with reduced glycemic impact and improved nutritional profile, suitable for integration into the diets of patients with diabetes

mellitus. The choice of ingredients was based on both their chemical composition and their technological and functional properties, which influence the structure of the final product and nutrient bioavailability.

As a reference product, refined wheat flour type 650 was used, which is specific to conventional bakery products. This type of flour is characterized by a high content of easily digestible starch and a low dietary fiber content, resulting in a high glycemic index and a pronounced postprandial glycemic response [4]. For these reasons, white bread was used as the control sample in the comparative study.

For the hypoglycemic formulations, flours with superior nutritional value were selected. Whole wheat flour was included due to its high content of dietary fiber, vitamins, and bioactive compounds, resulting from the preservation of the entire grain (bran, germ, and endosperm). The consumption of whole grains has been associated with improved insulin sensitivity and reduced risk of diabetes mellitus, effects mainly attributed to their fiber content and the complex structure of the food matrix [5].

Rye flour was selected as another key raw material, being recognized for its high content of soluble dietary fibers, particularly arabinoxylans, which contribute to increased intestinal viscosity and slower glucose absorption. Studies have demonstrated that rye-based products lead to a lower glycemic response compared to those obtained from refined flour, making them suitable for individuals with metabolic disorders [8].

Buckwheat flour, used in combination with chia seeds, was selected due to its pseudocereal nature and distinct nutritional composition. Buckwheat is rich in high-quality proteins, dietary fiber, and phenolic compounds, and exhibits a lower glycemic index compared to conventional cereals. Furthermore, its structural characteristics contribute to reduced starch digestibility, positively influencing glycemic response [8].

Chia seeds were included as a functional ingredient due to their high content of soluble fiber and polyunsaturated fatty acids. Soluble fibers have the ability to form viscous gels in the digestive tract, thereby slowing carbohydrate absorption and reducing postprandial glycemic fluctuations [6]. In addition, their incorporation contributes to improved texture and increased nutritional value of the final products.

All formulations also included basic ingredients specific to the bread-making process, namely baker's yeast, table salt, and potable water. Yeast was used for dough fermentation, a process that influences not only the organoleptic properties of the final product but also its digestibility, through modifications in starch and protein structure. Fermentation can contribute to glycemic index reduction by promoting organic acid formation and altering the food matrix [8].

Therefore, the selection of raw materials was carried out strategically in order to obtain bakery products characterized by reduced available carbohydrate content and increased dietary fiber intake, both of which are essential factors in modulating glycemic response and supporting dietary management of diabetes mellitus.

2.2 Qualitative and quantitative acceptance of raw materials

The data presented in Table 1 reveal significant variability in the nutritional composition of the selected raw materials. Whole grain flours and rye flour exhibit substantially higher dietary fiber content compared to refined wheat flour, while buckwheat flour provides a balanced macronutrient profile. Chia seeds stand out due to their exceptionally high fiber and lipid content, contributing to improved metabolic properties. In contrast, refined flour and sugar are characterized by a high proportion of rapidly digestible carbohydrates and minimal fiber content, which are associated with an increased glycemic response.

Table 1. Comparative nutritional composition of selected raw materials used in bakery products (per 100 g)

Raw material	Carbohydrates (g)	Dietary fiber (g)	Protein (g)	Lipids (g)	Energy (kcal)	Reference
Refined wheat flour	72.0	2.7	10.3	1.7	340	[17]
Whole wheat flour	72.6	12.2	13.7	1.9	339	[18]
Rye flour	77.2	13.7	8.4	1.9	359	[19]
Buckwheat flour	75.0	10.4	8.9	2.5	358	[20]
Chia seeds	42.0	34.0	17.0	31.0	486	[21]
Wheat bran	64.5	42.0	16.0	4.3	216	[22]
Vital wheat gluten	13.0	0.6	75.0	1.9	370	[22]
Seeds mix (avg.)	27.0	12.0	20.0	45.0	560	[22]
Yeast (fresh)	41.0	26.0	40.0	7.0	325	[22]
Olive oil	0	0	0	100	884	[22]
Sugar (brown)	100	0	0	0	380	[22]
Salt	0	0	0	0	0	
Water	0	0	0	0	0	

Note: Values are expressed per 100 g of raw material and were obtained from literature sources [17–22]. Minor variations may occur depending on the origin and processing conditions of the raw materials.

In order to further evaluate the role of the main cereal and pseudocereal flours used in the formulation of bakery products, a comparative analysis was performed focusing on their nutritional and functional characteristics. The selected flours differ significantly in terms of carbohydrate composition, dietary fiber content, protein level, and technological behavior, all of which influence both the processing performance and the metabolic impact of the final products.

These differences are particularly relevant in the context of developing hypoglycemic bakery products, where the quality of carbohydrates and the fiber content play a key role in modulating postprandial glycemic response. The comparative nutritional and functional characteristics of the selected flours are summarized in Table 2.

Table 2. Comparative nutritional and functional characterization of selected flours (per 100 g)

Parameter	**Refined wheat flour**	**Whole wheat flour**	**Rye flour**	**Buckwheat flour**	**Reference**
Carbohydrates (g)	72.0	72.6	77.2	75.0	[17–20]
Dietary fiber (g)	2.7	12.2	13.7	10.4	[17–20]
Protein (g)	10.3	13.7	8.4	8.9	[17–20]
Lipids (g)	1.7	1.9	1.9	2.5	[17–20]
Energy (kcal)	340	339	359	358	[17–20]
Fiber classification	Very low	High	Very high	Moderate-high	[17–20]
Carbohydrate quality	Refined	Complex	Complex	Complex	[17–20]
Glycemic potential	High	Moderate	Reduced	Reduced	[17–20]
Functional properties	Low	Good	Very good	Good	[17–20]
Technological behavior	Excellent (gluten-rich)	Good	Dense structure	Gluten-free	[17–20]

Note: Values are expressed per 100 g of flour. Nutritional data were obtained from literature sources [17–20] and represent average values, which may vary depending on the botanical origin, processing method, and degree of milling of the flours

The comparative analysis presented in Table 2 highlights significant differences in dietary fiber content among the selected flours, which represents a key factor in modulating glycemic response. Refined wheat flour contains a very low amount of fiber (2.7 g/100 g), whereas whole wheat and rye flours provide substantially higher levels, reaching 12.2 g/100 g and 13.7 g/100 g, respectively

Rye flour exhibits the highest dietary fiber content, suggesting a stronger capacity to reduce carbohydrate digestibility and attenuate postprandial glycemic response. Whole wheat flour offers a balanced nutritional profile, combining elevated fiber and protein levels with good technological performance. Buckwheat flour, with a moderate to high fiber content (10.4 g/100 g), contributes additional functional benefits due to its pseudocereal nature and reduced gluten content.

These differences are directly reflected in the nutritional quality of the final bakery products and support the use of whole grain and alternative flours in the formulation of hypoglycemic foods for dietary management of diabetes mellitus.

2.3 Recipe Formulation and Standardization of the Technological Process

The formulation of the experimental recipes was carried out with the aim of obtaining bakery products with reduced carbohydrate content and increased dietary fiber, while maintaining acceptable technological and sensory properties.

White bread (WB) formulated with refined wheat flour was used as the control sample. Hypoglycemic variants were developed by partially or totally replacing refined flour with rye flour (RB), whole wheat flour (WGB), and buckwheat flour combined with chia seeds (BBCh). This substitution strategy was designed to reduce rapidly digestible carbohydrates and increase dietary fiber content, with expected beneficial effects on glycemic response.

The composition of each product was established based on technological and nutritional considerations, taking into account the different hydration capacity and gluten-forming potential of the selected raw materials.

The standardized formulations of the analyzed bakery products are presented in Table 3.

Table 3. Ingredient composition of bakery products used in the study

Ingredient	**Unit of measurement**	**WB**	**RB**	**WGB**	**BBCh**
Main flour	g	1000 refined wheat flour	220 rye flour + whole wheat flour	500 whole wheat flour	250 buckwheat
Water	ml	600	500	500	360
Yeast	g	25	25	25	8
Salt	g	20	7	20	7
Oil	ml	100	45	30	-
Bran	g	-	50	-	-
Seeds mix	g	-	30	100	-
Chia seeds	g	-	-	-	20
Sugar	g	-	7	-	-
Gluten	g	-	20	-	-

Note: Ingredient quantities are expressed per batch and were adapted to ensure optimal dough consistency and product quality

The formulations presented in Table 3 highlight the progressive substitution of refined wheat flour with whole grain and alternative ingredients characterized by higher dietary fiber content and reduced digestible carbohydrates.

The RB and WGB formulations include bran and mixed seeds, contributing to an increase in fiber and protein content, while the BBCh sample incorporates buckwheat flour and chia seeds, providing a distinct nutritional profile with functional properties. In contrast, the WB formulation is primarily based on refined flour, resulting in a higher proportion of rapidly available carbohydrates.

These compositional differences are essential for explaining the variations observed in the nutritional values and potential glycemic response of the final products.

2.4 Technological Process for the Production of Functional Bakery Products

The technological process applied for the production of the bakery products was standardized in order to ensure reproducibility and comparability between experimental variants. A classical bread-making process was used, adapted to the specific characteristics of the raw materials, while maintaining constant processing parameters.

The technological scheme of the process is presented in Figure 2 and includes the following stages: raw material preparation, dosing, mixing and kneading, fermentation, division and shaping, final proofing, baking, cooling, and storage.

Raw materials were weighed according to the established formulations and conditioned prior to processing. Mixing and kneading were carried out using a two-stage process, consisting of initial dough formation followed by final kneading, in order to achieve proper gluten network development. Kneading time ranged between 8–12 minutes, depending on flour type, with dough temperature maintained at 24–26°C.

Fermentation was conducted using baker's yeast at controlled conditions of 28–30°C for 60–90 minutes. In samples containing whole grain and pseudocereal flours, fermentation time was slightly extended to compensate for reduced gluten content and lower gas retention capacity.

After primary fermentation, the dough was divided, shaped, and subjected to final proofing at 30–32°C and relative humidity of 75–85% for 30–60 minutes, until optimal volume development was achieved.

Baking was performed at temperatures between 180 and 220°C for 25–35 minutes, ensuring proper starch gelatinization and formation of a porous crumb structure. These transformations are essential for determining the digestibility of carbohydrates and the glycemic response of the final product.

Following baking, the products were cooled at room temperature (20–22°C) for 2–3 hours to stabilize the structure and prevent moisture condensation. The final products were stored under controlled conditions until further analysis.

The application of standardized technological parameters allowed for the isolation of the effect of formulation on product characteristics, ensuring the reliability of the experimental results.

The technological flow diagram illustrating the processing stages of the bakery products is presented in Figure 2.

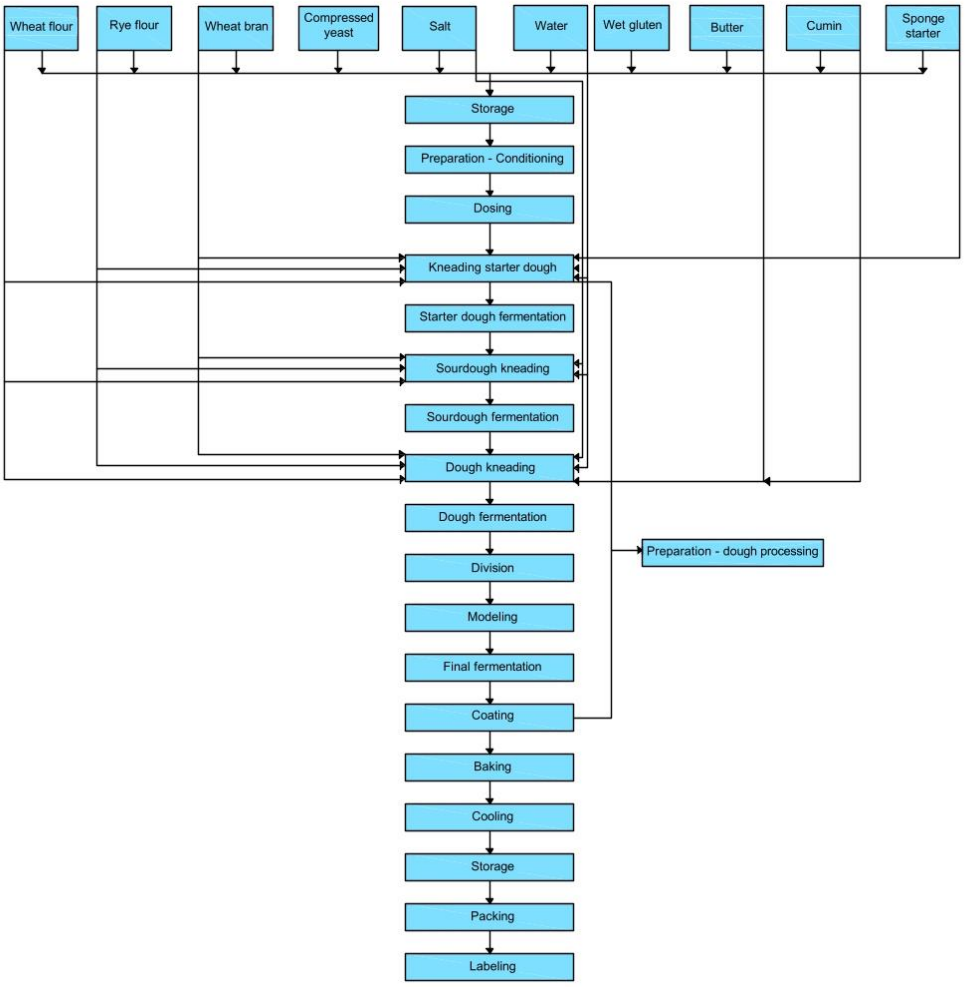

Figure 2. Technological flow diagram for the production of bakery products

The scheme highlights the sequence of technological operations and the integration of raw materials into the production process, ensuring process consistency and product quality.

2.5 Nutritional Analysis and Determination of Chemical Composition

The determination of macronutrient composition was performed according to standard analytical methods. Protein content was estimated using the Kjeldahl method (AOAC 920.87), while lipid content was determined by Soxhlet extraction (AOAC 922.06). Moisture content was evaluated according to AOAC 925.10 [10]

Carbohydrates were calculated by difference, by subtracting the sum of protein, lipid, ash, and moisture contents from 100%. Dietary fiber content was estimated based on the individual contribution of raw materials, considering their composition.

Nutritional values were calculated using the exact ingredient proportions for each recipe and expressed per 100 g of product. Energy value was determined using Atwater factors (4 kcal/g for proteins and carbohydrates, 9 kcal/g for lipids).

All analyses were performed in triplicate, and results were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA followed by Tukey's post-hoc test, with a significance level of $p < 0.05$.

2.6 Sensory Analysis of Functional Bakery Products

The sensory evaluation of the analyzed bakery products was performed in order to assess the impact of reformulation on organoleptic characteristics and overall consumer acceptability. This step is essential in validating functional food products, as compositional

modifications—particularly increased dietary fiber content and the use of alternative ingredients—may significantly influence the sensory properties of the final product [8].

The sensory analysis was carried out using the hedonic scoring method, in accordance with the ISO 6658:2017 standard, which establishes general principles for the sensory evaluation of food products. This method is widely applied in both the food industry and research for determining product acceptability and comparing organoleptic characteristics across different variants [11].

The evaluation panel consisted of twenty semi-trained assessors familiar with the main sensory attributes of bakery products. The assessors received prior training to ensure consistent identification and evaluation of key characteristics such as texture, taste, and aroma, thereby enhancing the objectivity and reproducibility of the results.

The evaluation was conducted under controlled conditions, in rooms with uniform lighting and constant temperature, in order to minimize the influence of external factors on sensory perception. The samples were presented anonymously and in randomized order, and the assessors were not provided with information regarding product composition or type, in order to avoid subjective bias.

The sensory attributes analyzed included external appearance, color, aroma, texture, taste, and overall acceptability. Each attribute was evaluated using a scoring scale from 1 to 5, where 1 represented the lowest score and 5 represented the highest score. Overall acceptability was calculated as the mean of the scores assigned to the evaluated attributes, allowing for a comparative analysis between product variants.

The obtained results were summarized and expressed as mean values, which were used to assess the differences in sensory acceptability among the analyzed products. Products that achieved high scores were considered suitable for consumption, indicating that reformulation did not negatively affect their organoleptic characteristics.

The integration of sensory analysis into the study methodology enabled the correlation of nutritional modifications with their impact on consumer perception, thereby contributing to the validation of the developed products' applicability. This approach is particularly important in the context of clinical nutrition, where the acceptability of food products directly influences patient adherence to dietary interventions.

RESULTS AND DISCUSSIONS

3.1 Results on nutritional value and their interpretation

The nutritional values determined for the analyzed bakery products are presented in Table 4, expressed as mean ± standard deviation (SD), calculated based on three independent batches. The differences between variants were statistically evaluated, and values followed by different letters indicate statistically significant differences ($p < 0.05$).

These results reflect the direct impact of formulation on the nutritional composition of the products, particularly in terms of carbohydrate content, dietary fiber levels, and total energy value.

Table 4. Nutritional value of bakery products (per 100g product)

Nutritional parameter	Unit of measurement	WB	RB	WGB	BBCh
Energy (kcal)	Kcal	247.09 ± 2.15^{a}	223.73 ± 1.94^{b}	216.77 ± 1.82^{c}	161.67 ± 1.50^{d}
Protein	g	6.03 ± 0.21^{a}	7.77 ± 0.25^{b}	8.80 ± 0.28^{c}	6.91 ± 0.24^{d}
Carbohydrates	g	41.13 ± 0.52^{a}	35.48 ± 0.47^{b}	31.55 ± 0.43^{c}	26.92 ± 0.39^{d}
Lipids	g	6.30 ± 0.30^{a}	10.10 ± 0.33^{b}	7.50 ± 0.26^{b}	2.88 ± 0.20^{c}
Fiber	g	1.01 ± 0.09^{a}	10.10 ± 1.94^{b}	6.20 ± 0.28^{c}	4.95 ± 0.22^{d}

Note: Values are expressed as mean ± standard deviation (SD). Different letters on the same row indicate statistically significant differences ($p < 0.05$)

Legend:
WB – White Bread (refined wheat bread, control sample)
RB – Rye Bread (bread formulated with rye flour)
WGB – Whole Grain Bread (bread formulated with whole wheat flour)
BBCh – Buckwheat Bread with Chia (bread formulated with buckwheat flour and chia seeds)

The carbohydrate content differed significantly among all samples ($p < 0.05$), decreasing progressively from WB to BBCh. The WB sample exhibited the highest level of available carbohydrates (41.13 g/100 g), while the BBCh sample showed the lowest value (26.92 g/100 g).

This gradual reduction reflects the impact of reformulation strategies, where refined flour was replaced with whole grain and pseudocereal-based ingredients. The statistically distinct groups (different letters across all samples) confirm that each formulation significantly modifies the carbohydrate profile.

From a metabolic perspective, this reduction is particularly relevant, as lower carbohydrate availability is associated with a reduced postprandial glycemic response and improved glycemic control.

Dietary fiber content showed a significant increase in all reformulated samples compared to WB ($p < 0.05$). The RB sample presented the highest fiber content (10.10 g/100 g), followed by WGB and BBCh.

The WB sample, characterized by a very low fiber content (1.01 g/100 g), differed significantly from all other samples, confirming its limited functional value in metabolic nutrition.

The elevated fiber content in RB and WGB is associated with the use of whole grain flours and bran fractions, while BBCh benefits from the contribution of chia seeds and buckwheat flour. From a physiological standpoint, dietary fiber plays a critical role in delaying carbohydrate digestion and absorption, thereby contributing to improved glycemic stability.

The protein content varied significantly among samples, with WGB showing the highest value (8.80 g/100 g), followed by RB, BBCh, and WB. This distribution indicates the superior nutritional contribution of whole grain formulations in terms of protein intake.

Regarding lipids, RB and WGB exhibited similar values (no significant difference between them), while BBCh showed a significantly lower lipid content (2.88 g/100 g). The reduced lipid fraction in BBCh contributes to its lower energy density and enhances its suitability for hypocaloric diets.

The energy value showed statistically significant differences across all samples ($p < 0.05$), with a clear decreasing trend from WB (247.09 kcal/100 g) to BBCh (161.67 kcal/100 g).

To provide a clearer visualization of the nutritional changes induced by reformulation, the relative variation in carbohydrate and dietary fiber content compared to the control sample (WB) is presented in Figure 3 a)-b).

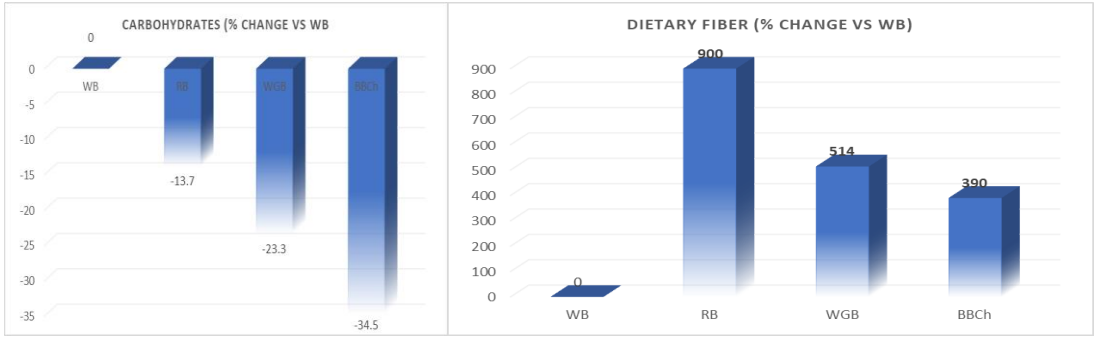

Figure 3. Relative variation (%) in carbohydrate content (a) and dietary fiber (b) of reformulated bakery products compared to the control sample (WB)

As illustrated in Figure 3 a), a consistent reduction in carbohydrate content was observed across all reformulated samples relative to the control. Compared to WB, carbohydrate levels decreased by approximately 13.7% in RB, 23.3% in WGB, and 34.5% in BBCh.

This progressive decrease reflects the impact of replacing refined flour with whole grain and pseudocereal-based ingredients, which contain lower proportions of rapidly digestible carbohydrates. From a metabolic perspective, this reduction is particularly significant, as it is directly associated with a lower postprandial glycemic response and improved glycemic control. The most pronounced reduction was observed in the BBCh sample, highlighting its potential as a hypoglycemic product.

In contrast, dietary fiber content showed a marked increase in all reformulated variants, as shown in Figure 3 b). The RB sample exhibited the highest increase, with an approximately nine-fold rise (+900%) compared to the control. Similarly, WGB and BBCh displayed substantial increases of approximately 514% and 390%, respectively.

This significant enhancement in fiber content is attributed to the use of whole grain flours, bran fractions, and functional ingredients such as chia seeds. From a physiological standpoint, increased dietary fiber intake is associated with delayed carbohydrate digestion, reduced glucose absorption rate, and improved glycemic stability.

Taken together, the graphical analysis demonstrates a clear and complementary trend: a reduction in carbohydrate availability alongside a substantial increase in dietary fiber content. This dual modification represents a major nutritional advantage of the reformulated products, as both factors contribute synergistically to improved metabolic outcomes.

Therefore, the results confirm that reformulation strategies significantly improve the nutritional quality of bakery products, supporting their use as effective dietary tools for glycemic control in diabetes management.

In order to further evaluate the nutritional impact of the analyzed bakery products, the contribution of 100 g of each sample to the recommended daily intake (RDI) of macronutrients was calculated. This parameter provides a practical perspective on the nutritional relevance of the products in the context of daily dietary requirements. The results are illustrated in Figure 4.

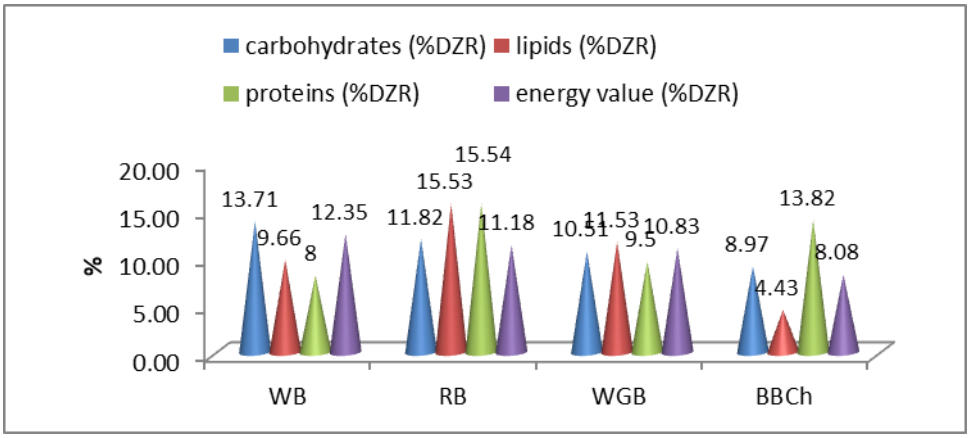

Figure 4. Contribution of 100 g of bakery products to recommended daily intake of macronutrients (%RDI)

As illustrated in Figure 4, the analyzed bakery products show distinct contributions to daily macronutrient intake, reflecting differences in formulation and raw material composition. The hypoglycemic variants demonstrate a reduced contribution to carbohydrate intake compared to white bread, along with a more balanced distribution of proteins and

lipids. These modifications indicate an improved nutritional profile, supporting the role of reformulated bakery products in dietary strategies aimed at glycemic control and metabolic health.

The evaluation of the contribution of 100 g of bread products to the recommended daily intake (RDI) highlights important nutritional differences among the analyzed formulations, emphasizing the impact of flour type and functional ingredients on the nutritional profile of bakery products. The percentages were calculated considering the average adult daily requirements of 50 g proteins, 65 g lipids, and 300 g carbohydrates, corresponding to an energy intake of approximately 2000–2500 kcal/day [20]. These values are in agreement with European dietary reference frameworks proposed by EFSA for macronutrient intake in adults.

Among the analyzed samples, White Bread (WB) exhibited the highest contribution to carbohydrate intake, providing 13.71% of the daily carbohydrate requirement per 100 g product. This observation is consistent with the high proportion of refined wheat flour, characterized by increased starch availability and lower dietary fiber content. In contrast, the lipid contribution remained relatively low (9.66% DZR), while the protein contribution reached 8.70% DZR. The energetic contribution of WB represented 12.35% of the daily energy requirement, indicating a predominantly carbohydrate-based nutritional profile [21-23].

Rye Bread (RB) demonstrated the most balanced nutritional distribution and the highest overall nutritional density among the investigated products. The protein contribution reached 15.54% DZR, representing the highest value recorded among all samples, while lipid contribution increased to 15.53% DZR. In parallel, RB provided 11.82% of the recommended carbohydrate intake and 11.18% of the daily energy requirement. The elevated protein and lipid contributions may be associated with the higher nutritional complexity of rye flour, which contains increased levels of bioactive compounds, dietary fiber, and structurally complex carbohydrates. Rye-based products are frequently associated with improved satiety and attenuated glycemic responses due to slower starch digestion kinetics and enhanced matrix viscosity [11,12].

Whole Grain Bread (WGB) showed intermediate nutritional characteristics, with carbohydrate contribution values of 10.11% DZR and lipid contribution of 9.53% DZR. However, the protein contribution remained relatively high (10.80% DZR), reflecting the preservation of bran and germ fractions in whole wheat flour. The energetic contribution reached 10.83% DZR. Whole grain products are recognized for their higher dietary fiber, mineral, and phytochemical content compared to refined products, contributing to improved metabolic regulation and reduced postprandial glycemic variability [13]. Moreover, the structural integrity of whole grain matrices may limit enzymatic starch hydrolysis and delay glucose release during digestion [24,25]

Buckwheat Bread with Chia (BBCh) presented the lowest carbohydrate contribution among all formulations (8.97% DZR), while simultaneously exhibiting the highest energetic contribution (13.82% DZR). The lipid contribution remained comparatively low (4.43% DZR), whereas protein intake accounted for 8.08% DZR. The reduced carbohydrate contribution may be attributed to the replacement of refined cereal flour with buckwheat and chia ingredients, both characterized by higher dietary fiber content and lower digestible starch fractions. Chia seeds additionally contribute functional lipids rich in polyunsaturated fatty acids and bioactive compounds associated with metabolic health benefits. Such formulations are particularly relevant in the context of low-glycemic dietary strategies and functional food development [26-27].

Overall, the results demonstrate that the reformulation of bread products through the incorporation of whole grains, rye flour, buckwheat, and chia seeds significantly modifies the nutritional contribution of bakery products to daily dietary requirements. These changes may

contribute to improved nutritional quality, enhanced satiety potential, and better metabolic control, supporting the growing interest in functional bakery products designed for the prevention and dietary management of metabolic disorders. Similar observations regarding the metabolic advantages of whole grain and fiber-enriched bakery products have been extensively reported in recent nutritional and clinical studies.

3.2 Results of sensory analysis and their interpretation

The results of the sensory analysis for each type of bakery product are presented in Table 5. The scores were obtained based on the evaluation performed by a panel of semitrained assessors, under controlled conditions, using the hedonic scoring method.

Table 5. Sensory evaluation scores of bakery products

Sensory Feature	WB	RB	WGB	BBCh
External appearance	4.5 ± 0.2^{a}	4.2 ± 0.2^{b}	4.3 ± 0.2^{b}	4.4 ± 0.3^{a}
Color	4.6 ± 0.2^{a}	4.1 ± 0.3^{b}	4.2 ± 0.2^{b}	4.3 ± 0.3^{b}
Smell/Aroma	4.4 ± 0.3^{a}	4.2 ± 0.2^{b}	4.3 ± 0.2^{ab}	4.2 ± 0.3^{b}
Texture/Consistency	4.6 ± 0.2^{a}	4.1 ± 0.3^{b}	4.3 ± 0.2^{c}	4.0 ± 0.3^{d}
Taste	4.5 ± 0.2^{a}	4.2 ± 0.3^{b}	4.3 ± 0.2^{b}	4.1 ± 0.3^{c}
Overall acceptability	4.52 ± 0.18^{a}	4.16 ± 0.21^{b}	4.28 ± 0.17^{ab}	4.20 ± 0.22^{b}

Note: Values are expressed as mean ± standard deviation (SD). Different letters on the same row indicate statistically significant differences ($p < 0.05$)

Legend:
WB – White Bread (refined wheat bread, control sample)
RB – Rye Bread (bread formulated with rye flour)
WGB – Whole Grain Bread (bread formulated with whole wheat flour)
BBCh – Buckwheat Bread with Chia (bread formulated with buckwheat flour and chia seeds

The data presented in Table 5 reveal statistically significant differences between the analyzed samples, indicating that the formulation of bakery products influences sensory perception across multiple attributes.

The WB sample achieved the highest scores for most parameters, particularly for color, texture, and taste, reflecting the well-established sensory properties of refined wheat-based products. These characteristics are mainly attributed to the optimal gluten structure and high consumer familiarity with white bread.

However, despite its superior sensory profile, WB does not represent a nutritionally optimal choice, as previously demonstrated by its high carbohydrate content and low fiber level.

The reformulated products (RB, WGB, and BBCh) exhibited high sensory acceptability, with all overall acceptability scores exceeding 4.0, indicating good consumer acceptance despite modifications in formulation.

The WGB sample demonstrated a balanced sensory profile, with consistently high scores across all attributes and no significant differences compared to WB in certain characteristics such as aroma and overall acceptability. This suggests that whole grain incorporation can preserve desirable sensory properties while improving nutritional value.

The RB sample, characterized by the highest fiber content, showed slightly lower scores for texture and color. These differences may be attributed to the denser structure and darker appearance typical of rye-based products. Nevertheless, its overall acceptability remained high, indicating that these differences do not significantly impair consumer acceptance.

The BBCh sample presented lower scores for texture and taste compared to WB and WGB, with statistically significant differences ($p < 0.05$). This effect is primarily related to the absence of gluten in buckwheat and the inclusion of chia seeds, which alter the structure and mouthfeel of the product.

Despite these differences, the overall acceptability score remained above 4.0, confirming that the product is still well accepted. Considering that BBCh also exhibited the most favorable nutritional profile, this result highlights its potential as a functional food suitable for dietary interventions.

The radar (spider) chart (Figure 5) provides a visual representation of the sensory profiles of the analyzed samples, allowing simultaneous comparison of all evaluated attributes. The graphical representation clearly shows that WB occupies the largest area, confirming its superior overall sensory performance. WGB presents a relatively large and well-balanced area, indicating a consistent sensory profile across all evaluated attributes.

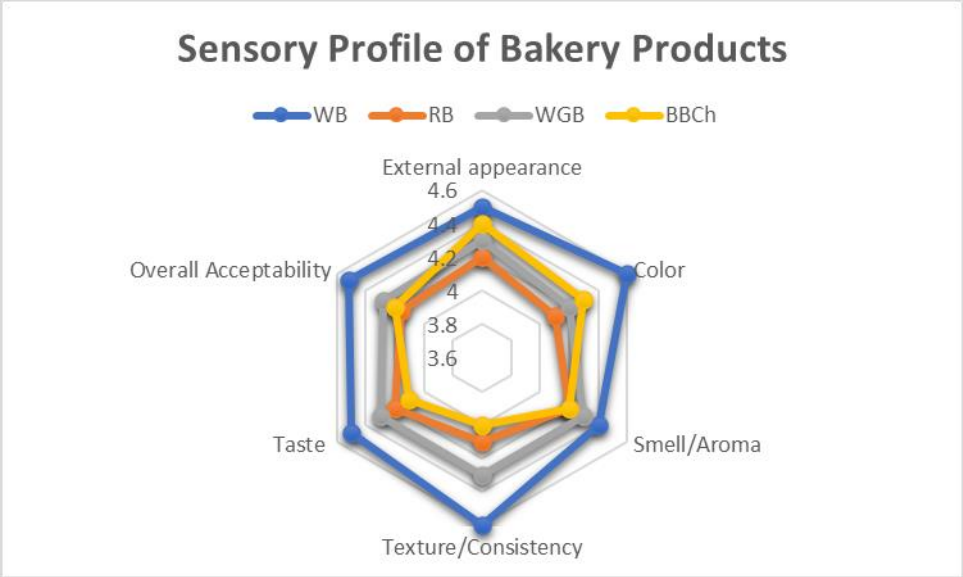

Figure 5. Radar chart representation of the sensory profile of bakery products (WB, RB, WGB, BBCh)

In contrast, RB exhibits a slightly reduced profile, particularly in terms of color and texture, while BBCh displays the smallest area, mainly due to lower scores recorded for texture and taste. However, the differences between samples remain relatively small, and all products maintain a compact radar profile, indicating good overall sensory quality.

This visual representation (Figure 5) confirms that reformulated bakery products retain acceptable sensory characteristics despite compositional modifications, supporting their potential application in dietary interventions targeting metabolic disorders.

From a clinical perspective, the sensory acceptability of functional foods is critical for long-term dietary adherence, especially in the management of chronic conditions such as diabetes mellitus.

The visual appearance of the analyzed bakery products is presented in Figure 7. a)-d), highlighting the differences in color, structure, and morphological characteristics between the samples. The WB sample exhibits a typical light color and uniform crumb structure, characteristic of refined wheat bread. In contrast, the RB and WGB variants show a darker color and a denser crumb, reflecting the presence of whole grain components and higher fiber content. The BBCh sample presents the most compact structure and darker appearance, associated with the absence of gluten and the incorporation of buckwheat flour and chia seeds. These visual differences are consistent with the compositional variations of the raw materials and support the results obtained in the sensory and nutritional analyses.

a) WB

b) RB

c) WGB

d) BBCh

Figure 6. a)-d) Visual appearance of bakery products illustrating differences in color, structure, and surface characteristics: (a) WB, (b) RB, (c) WGB, and (d) BBCh.

The results demonstrate that all reformulated samples maintain high acceptability scores, suggesting that nutritional improvements (e.g., increased fiber and reduced carbohydrate content) do not compromise palatability.

This aspect is particularly important in medical nutrition therapy, where patient compliance is directly influenced by the sensory properties of recommended foods. The ability to develop

The combined analysis of sensory and nutritional results indicates that:

- WB provides the highest sensory quality but the least favorable metabolic profile
- WGB offers the best balance between sensory performance and nutritional improvement
- RB provides superior fiber content with acceptable sensory characteristics
- BBCh presents the most advantageous metabolic profile with slightly reduced, but still acceptable, sensory scores

Overall, the results support the feasibility of developing hypoglycemic bakery products that maintain consumer acceptability, highlighting the importance of integrating sensory evaluation into the design of functional foods for clinical nutrition.

products that combine metabolic benefits with acceptable taste and texture represents a key factor in the success of dietary interventions.

CONCLUSIONS

In conclusion, the findings of the present study demonstrate that the reformulation of bakery products represents a viable and clinically relevant strategy for improving dietary quality in patients with carbohydrate metabolism disorders, particularly diabetes mellitus. The observed reduction in available carbohydrates, combined with the substantial increase in dietary fiber content, provides a metabolic profile that is closely aligned with current nutritional recommendations for glycemic control.

The analysis of the contribution of the investigated bread formulations to the recommended daily intake values demonstrated that the incorporation of rye flour, whole grains, buckwheat flour, and chia seeds significantly influenced the nutritional profile of the bakery products. Reformulated breads exhibited improved nutritional density through enhanced protein contribution, moderated carbohydrate availability, and more balanced energy distribution compared to conventional white bread formulations.

Among the analyzed samples, rye bread presented the highest protein and lipid contributions, suggesting an increased nutritional complexity and potential satiety-enhancing effect. Whole grain bread provided a balanced macronutrient profile associated with the preservation of fiber-rich cereal fractions, while buckwheat bread with chia seeds showed the lowest carbohydrate contribution, supporting its potential suitability for low-glycemic dietary strategies. In contrast, white bread remained predominantly carbohydrate-oriented, reflecting the typical nutritional characteristics of refined cereal products.

These findings support the growing evidence that reformulation of staple bakery products using functional and whole-grain ingredients may improve their nutritional and metabolic relevance. Such products may contribute to better glycemic management, enhanced dietary quality, and increased alignment with contemporary recommendations for the prevention and nutritional management of metabolic disorders.

Importantly, the maintained sensory acceptability of the reformulated products supports their practical applicability in medical nutrition therapy, where long-term adherence remains a critical determinant of therapeutic success. This dual achievement—metabolic improvement without compromise in palatability—highlights the potential of functional bakery products as effective dietary tools in clinical settings.

From a translational perspective, these results support the integration of reformulated staple foods into evidence-based nutritional strategies for diabetes management, contributing to the development of sustainable, patient-centered interventions. The present study is therefore highly relevant for clinical nutrition practice and supports the growing paradigm of "food as medicine" in metabolic disease management, aligning with the scope and objectives of journals focused on endocrinology, diabetes, and nutritional sciences.

Patents

No patents resulted from the work reported in this manuscript.

Acknowledgments

The authors would like to thank to the Doctoral School "Engineering of Vegetable and Animal Resources", University of Life Sciences "King Mihai I" from Timişoara (Calea Aradului 119, 300645 Timişoara, Romania), for the academic support and resources provided throughout the development of this research.

The authors used ChatGPT-5 (OpenAI, San Francisco, CA, USA), an artificial intelligence language model developed by OpenAI, exclusively for graphical design support and language refinement. All scientific interpretation and validation were performed by the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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Clinical Effects of Oral Probiotics on Plaque Accumulation and Gingival Inflammation in Young Adults

https://doi.org/10.70921/medev.v32i2.2036

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Received: 05 June 2026; Accepted: 13 June 2026; Published: 30 June 2026

Abstract

Background/Objectives: Oral dysbiosis is recognized as a major contributing factor to the development of biofilm-associated oral diseases, including dental caries and gingivitis. Probiotics have emerged as promising adjunctive agents capable of modulating the oral microbiota and supporting oral health. The aim of this study was to evaluate the effects of a chewable multi-strain probiotic formulation on salivary pH, plaque accumulation, and gingival inflammation in healthy young adults. Methods: Thirty participants aged 18–25 years were allocated to a probiotic group (n = 15) or a control group (n = 15) and monitored for 28 days. The probiotic group received chewable tablets containing *Streptococcus salivarius*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, and *Lactobacillus paracasei* for 14 days, followed by a 14-day washout period. Salivary pH, Plaque Index (PI), and Gingival Index (GI) were assessed at baseline, Day 14, and Day 28. Results: No significant differences in salivary pH were observed between groups throughout the study period ($p > 0.05$). In contrast, probiotic supplementation resulted in significantly lower PI values at Day 14 ($p = 0.042$) and significantly lower GI values at Day 14 ($p = 0.020$) and Day 28 ($p = 0.033$) compared with the control group. Within-group analyses also demonstrated significant reductions in plaque accumulation and gingival inflammation following probiotic administration. Conclusion: Short-term supplementation with a multi-strain probiotic formulation improved plaque control and gingival health without significantly affecting salivary pH, supporting the potential role of probiotics as adjunctive tools in preventive oral healthcare.

Keywords: probiotics; oral microbiome; salivary pH; plaque index; gingival index; oral health; preventive dentistry

INTRODUCTION

The oral cavity represents one of the most diverse microbial habitats in the human body, harboring a highly complex ecosystem composed of bacteria, fungi, viruses, and protozoa that coexist in a dynamic equilibrium with the host. Following the gastrointestinal tract, the oral environment contains one of the largest and most diverse microbial communities, with more than 700 bacterial species identified to date. These microorganisms colonize various oral surfaces, including teeth, gingival tissues, the tongue, buccal mucosa, and saliva, forming highly organized biofilms that play a fundamental role in maintaining oral and systemic health [1].

Oral biofilms are structured microbial communities embedded within a self-produced extracellular polymeric matrix and attached to biological or inert surfaces. Unlike planktonic microorganisms, bacteria within biofilms exhibit complex interactions that allow them to adapt to environmental fluctuations, regulate local pH and redox potential, and establish cooperative or competitive relationships with neighboring species. Under physiological conditions, these microbial communities contribute to maintaining oral homeostasis by preventing pathogen colonization, modulating immune responses, and supporting the integrity of oral tissues. Consequently, the oral microbiome should not be regarded merely as a collection of microorganisms but rather as an essential component of the oral ecosystem that actively participates in health maintenance [1].

The healthy oral microbiota is characterized by a balanced composition of commensal microorganisms capable of limiting the growth of pathogenic species. Beneficial bacterial genera, including Lactobacillus and Bifidobacterium, have been identified as important contributors to microbial stability and host protection. Studies based on culture-dependent and molecular techniques have demonstrated that these microorganisms colonize the oral cavity early in life, partly through maternal transmission and exposure to breast milk. Species such as Bifidobacterium bifidum, Bifidobacterium dentium, and Bifidobacterium longum have been isolated from oral samples and are generally associated with beneficial effects on host health. These microorganisms contribute to the maintenance of ecological balance by competing with pathogens for nutrients and adhesion sites while simultaneously supporting immune regulation [2].

Despite the resilience of the oral microbiome, numerous endogenous and exogenous factors can disrupt this delicate balance and promote dysbiosis. Oral dysbiosis refers to qualitative and quantitative alterations in microbial composition that favor the proliferation of pathogenic microorganisms and increase susceptibility to disease. The transition from health to disease is not generally associated with the emergence of a single pathogen but rather with ecological shifts involving multiple microbial species and changes in their metabolic activity. Such alterations may result in the development of common oral diseases, including dental caries, gingivitis, and periodontitis, which continue to represent significant public health concerns worldwide [3] .

Dental caries remains one of the most prevalent chronic diseases globally and affects individuals across all age groups. According to the ecological plaque hypothesis, frequent exposure to fermentable carbohydrates promotes environmental acidification, resulting in selective pressure favoring acidogenic and aciduric bacterial species such as Streptococcus mutans and various Lactobacillus species. This shift toward an acidic microbiome suppresses beneficial microorganisms that thrive under neutral pH conditions and contributes to progressive demineralization of dental tissues. Similarly, gingivitis and periodontal diseases are associated with dysbiotic changes characterized by increased bacterial load, altered microbial composition, and persistent inflammatory responses within periodontal tissues [1].

The development of oral dysbiosis is influenced by a broad range of factors. Prolonged or inappropriate antibiotic therapy may alter microbial diversity and facilitate the emergence of opportunistic pathogens. Dietary habits, particularly high consumption of refined sugars and processed carbohydrates, can significantly affect microbial metabolism and ecological balance. Additional factors such as smoking, alcohol consumption, hormonal changes, systemic diseases, stress, geographical variations, genetic predisposition, and inadequate oral hygiene practices further contribute to microbial disruption and disease susceptibility. Emerging evidence has also demonstrated that psychological stress may influence microbial diversity and community structure, potentially increasing vulnerability to oral diseases through both behavioral and biological pathways [3,4].

Given the multifactorial nature of oral diseases and the increasing concerns regarding antimicrobial resistance, interest has grown in alternative and adjunctive approaches capable of promoting oral health without disrupting microbial balance. Among these strategies, probiotics have emerged as particularly promising candidates. The World Health Organization defines probiotics as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. Although traditionally associated with gastrointestinal health, probiotics have increasingly been investigated for their potential applications in oral medicine and dentistry [5].

The rationale for probiotic use in oral health is based on their capacity to modulate microbial communities and influence host immune responses. Rather than eliminating microorganisms indiscriminately, probiotics contribute to restoring ecological balance by promoting beneficial bacterial populations and suppressing pathogenic species. This ecological approach is particularly attractive because it aligns with contemporary concepts of microbiome-centered disease prevention and management. As a result, probiotics are increasingly viewed as biological agents capable of supporting oral health through mechanisms that extend beyond simple antimicrobial activity [5].

One of the most extensively studied properties of probiotics is their anti-inflammatory potential. Several Lactobacillus and Bifidobacterium strains have demonstrated the ability to modulate both innate and adaptive immune responses. These microorganisms can stimulate immunoglobulin A production, regulate cytokine secretion, and reduce the expression of pro-inflammatory mediators such as interleukin-1β and tumor necrosis factor-alpha. Through these mechanisms, probiotics may contribute to reducing gingival inflammation and preserving periodontal tissue integrity. Furthermore, by promoting epithelial repair and strengthening mucosal barriers, probiotic bacteria help maintain host–microbe homeostasis and protect oral tissues from inflammatory damage [5].

In addition to their immunomodulatory effects, probiotics possess significant antimicrobial properties. Various probiotic strains produce organic acids, hydrogen peroxide, bacteriocins, and other antimicrobial peptides capable of inhibiting the growth of pathogenic microorganisms. They also compete with pathogens for adhesion sites and nutrients, thereby reducing colonization and biofilm formation. These mechanisms are particularly relevant in the oral cavity, where biofilm accumulation plays a central role in the pathogenesis of both caries and periodontal diseases. By influencing microbial composition and biofilm dynamics, probiotics may contribute to a healthier oral environment and reduced disease risk [5].

Another important characteristic of probiotics is their antioxidant activity. Oxidative stress has been increasingly recognized as a contributing factor in the pathogenesis of oral inflammatory diseases. Reactive oxygen species generated during chronic inflammation may damage host tissues and perpetuate disease progression. Probiotic microorganisms have demonstrated the capacity to reduce oxidative stress through multiple pathways, including the production of antioxidant enzymes, enhancement of antioxidant absorption, modulation

of inflammatory mediators, and regulation of microbial metabolism. Such effects may provide additional benefits in maintaining oral tissue health and preventing disease progression [2].

Recent investigations have specifically focused on the influence of probiotics on salivary characteristics and clinical oral health parameters. Saliva plays a crucial role in maintaining oral homeostasis through its buffering capacity, antimicrobial properties, and contribution to remineralization processes. Alterations in salivary pH may significantly affect microbial ecology and caries susceptibility. Several studies have suggested that probiotic supplementation can enhance salivary pH and buffering capacity by promoting alkali-generating metabolic pathways involving compounds such as arginine and urea. These processes contribute to the production of ammonia, favoring a less acidic environment and supporting the maintenance of ecological balance within the oral cavity [6–9].

Furthermore, clinical trials have demonstrated beneficial effects of probiotic supplementation on plaque accumulation and gingival inflammation. Significant reductions in plaque index and gingival index scores have been reported following the administration of probiotic tablets or lozenges, suggesting that probiotics may serve as valuable adjuncts to conventional oral hygiene measures. Particular attention has been directed toward strains such as Lactobacillus reuteri, Lactobacillus paracasei, and Streptococcus salivarius K12 and M18, which have shown promising results in reducing oral biofilm formation, decreasing gingival inflammation, and inhibiting cariogenic bacteria, including Streptococcus mutans [6,8,10–12].

Despite the growing body of evidence supporting the beneficial effects of probiotics in oral health, significant heterogeneity remains regarding probiotic strains, administration protocols, treatment duration, and clinical outcomes. Moreover, studies involving young adults remain relatively limited, particularly in Eastern European populations. Therefore, additional research is needed to clarify the clinical relevance of probiotic supplementation and its impact on key oral health parameters [1,2].

Maintaining a balanced oral microbiome has emerged as a central objective in contemporary preventive dentistry, given its fundamental role in preserving oral homeostasis and preventing biofilm-associated diseases. While conventional preventive approaches primarily focus on mechanical plaque control and antimicrobial agents, increasing attention has been directed toward probiotic supplementation as a strategy capable of modulating the oral ecosystem without disrupting its natural microbial equilibrium. Despite the growing body of evidence supporting the beneficial effects of probiotics on oral health, the extent to which these microorganisms influence clinically relevant parameters such as salivary pH, plaque accumulation, and gingival inflammation remains incompletely understood, particularly in young adults with generally good oral health status.

Although several clinical trials have investigated the effects of probiotics on oral health outcomes, the available evidence remains inconsistent. Variations in probiotic strains, treatment duration, dosage, and study populations have produced heterogeneous results regarding changes in salivary pH, plaque accumulation, and gingival inflammation [13,14]. Moreover, relatively few studies have focused on healthy young adults, a population in which early preventive interventions may contribute to the long-term maintenance of oral health. Consequently, additional clinical investigations are required to better understand the effectiveness of probiotic supplementation as a microbiome-modulating strategy in preventive dentistry [13,15–17].

Aim and objectives

The present study was undertaken to evaluate the effects of a chewable probiotic formulation containing Streptococcus salivarius, Lactobacillus acidophilus, Lactobacillus

paracasei, and other beneficial probiotic strains on selected parameters associated with oral health in young adults. Growing interest in microbiome-based approaches for disease prevention has highlighted the importance of probiotics as potential modulators of the oral ecosystem, capable of influencing both microbial composition and host-related factors involved in the maintenance of oral health. In this context, the study focused on assessing the impact of probiotic administration on salivary pH, a key determinant of the oral environment that plays a crucial role in the balance between demineralization and remineralization processes, as well as on clinical indicators of oral hygiene and periodontal health, including the Plaque Index and Gingival Index.

The investigation sought to determine whether regular consumption of the probiotic supplement could contribute to the stabilization of salivary conditions, reduce the accumulation of dental biofilm, and diminish gingival inflammatory responses. These parameters were selected due to their well-established association with the development and progression of common oral diseases, including dental caries and gingivitis, and because they provide clinically relevant information regarding the effectiveness of preventive interventions aimed at improving oral health outcomes.

Beyond the evaluation of individual clinical endpoints, the study was designed to explore the broader implications of probiotic supplementation in supporting oral microbial homeostasis. By promoting the growth and activity of beneficial microorganisms while limiting the proliferation of potentially pathogenic species, probiotics may contribute to the ecological balance of the oral cavity and enhance its resilience against disease-associated dysbiosis. Consequently, the present research aimed to provide a comprehensive assessment of both biological and clinical responses to probiotic administration, offering further insight into the potential role of probiotics as a non-invasive, preventive strategy for maintaining oral health. Through the integration of salivary and periodontal parameters, the findings may contribute to the expanding body of evidence supporting the incorporation of microbiome-oriented approaches into contemporary preventive dentistry and oral healthcare practices.

MATERIALS AND METHODS

This controlled clinical trial was conducted to evaluate the effects of oral probiotic supplementation on salivary pH, plaque accumulation, and gingival inflammation in young adults. The study included 30 participants aged between 18 and 25 years, who were recruited after providing written informed consent. Participants were allocated into two parallel groups consisting of 15 individuals each and were monitored over a 28-day period. The intervention period consisted of 14 days of probiotic administration, followed by a 14-day observation period.

The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Victor Babeș University of Medicine and Pharmacy Timișoara (Approval No. 103/03.09.2024 rev2026). Written informed consent was obtained from all participants prior to enrollment.

Eligible participants were healthy young adults without severe chronic or autoimmune diseases and willing to comply with the study protocol. Exclusion criteria included antibiotic therapy within the previous four weeks, current use of probiotic supplements, pregnancy or breastfeeding, known hypersensitivity to probiotic ingredients, and a history of periodontal treatment involving scaling and root planing before enrollment.

Participants assigned to the test group received chewable probiotic tablets (Swanson® Oral Probiotic Formula, Swanson Health Products, Fargo, ND, USA) containing a proprietary blend of 3 billion colony-forming units (CFU) per serving. The formulation included BLIS K12 Streptococcus salivarius, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus

reuteri, and Lactobacillus paracasei. BLIS K12 is a patented strain of Streptococcus salivarius specifically selected for its potential benefits in oral health maintenance. Participants were instructed to chew and slowly dissolve one tablet daily in the oral cavity immediately after evening tooth brushing for 14 consecutive days. Food and beverage consumption was prohibited after tablet administration.

The control group did not receive probiotic supplementation and was instructed to maintain its regular oral hygiene routine, including tooth brushing twice daily throughout the study period. No additional dietary restrictions or oral hygiene interventions were introduced in either group.

Clinical and salivary assessments were performed at baseline (Day 0), after completion of probiotic administration (Day 14), and at the end of the follow-up period (Day 28). The primary outcomes included salivary pH, Plaque Index (PI), and Gingival Index (GI).

Unstimulated whole saliva samples were collected under standardized conditions. Participants were instructed to refrain from eating, drinking, smoking, chewing gum, or performing oral hygiene procedures for at least two hours before sample collection. Salivary pH was measured immediately after collection using a calibrated digital pH meter according to the manufacturer's recommendations.

Dental plaque accumulation was assessed using the Silness–Löe Plaque Index (PI), which evaluates the thickness of plaque deposits along the gingival margin on selected tooth surfaces. Gingival inflammation was evaluated using the Löe–Silness Gingival Index (GI), based on visual signs of inflammation and bleeding tendency following gentle probing. All clinical examinations were performed using standardized assessment criteria in order to ensure consistency throughout the study period.

Data were recorded in a dedicated database and analyzed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). Descriptive statistics were calculated for all variables and are presented as mean ± standard deviation (SD). Comparisons between groups were performed using the independent-samples Student's t-test, whereas longitudinal comparisons within the same group were analyzed using the paired Student's t-test. Statistical significance was established at a p-value < 0.05.

RESULTS

Salivary pH represents an important indicator of oral environmental balance, influencing enamel integrity, microbial composition, and the susceptibility to dental caries. Throughout the study period, salivary pH values remained within the physiological range in both the probiotic and control groups, suggesting the maintenance of a favorable oral environment.

At baseline, the mean salivary pH was 6.75 ± 0.42 in the probiotic group and 6.65 ± 0.21 in the control group. Following the 14-day intervention period, a slight increase in salivary pH was observed in both groups, reaching 6.82 ± 0.21 in the probiotic group and 6.80 ± 0.25 in the control group. At the end of the follow-up period (Day 28), the mean salivary pH values were 6.77 ± 0.30 and 6.71 ± 0.15, respectively (Table 1).

Table 1. Comparison of salivary pH values between the probiotic and control groups during the study period

		Day 0± SD	**Day 14 ± SD**	**Day 28 ± SD**
Probiotic group	Mean ± SD	6,75± 0,42	6,82± 0,21	6,77± 0,3
	N	15	15	15
Control grup	Mean ± SD	6,65± 0,21	6,80± 0,25	6,77± 0,15
	N	15	15	15
p-value		0,457	0,814	1,000

Although minor fluctuations were observed over time, salivary pH remained relatively stable in both groups. Statistical analysis revealed no significant differences between the probiotic and control groups at any of the evaluated time points (Day 0: p = 0.457; Day 14: p = 0.814; Day 28: p = 1.000), indicating that probiotic supplementation did not produce a measurable effect on salivary pH under the conditions of the present study.

The temporal evolution of salivary pH values in the probiotic and control groups is illustrated in Figure 1. As shown, both groups exhibited similar trends throughout the study period, with values remaining within the range generally associated with oral health and ecological stability.

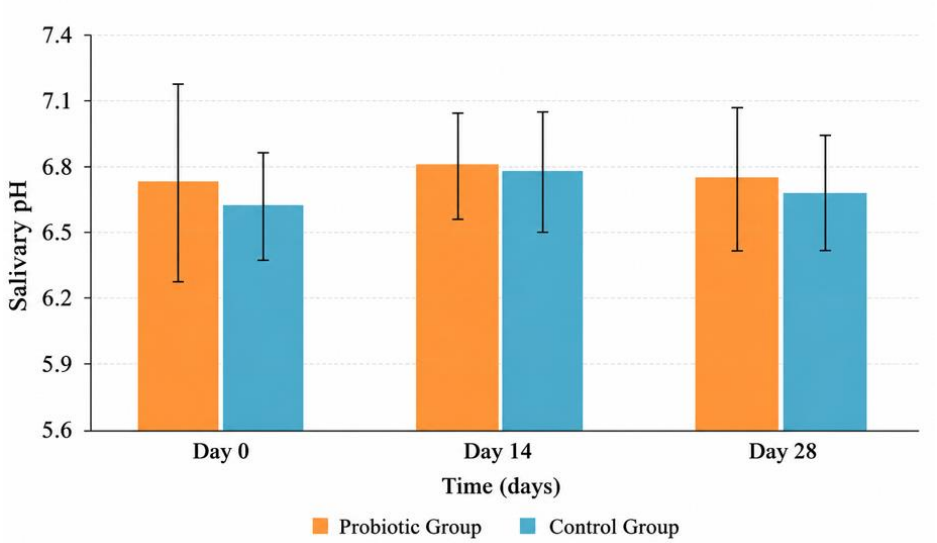

Figure 1. Temporal evolution of salivary pH values in the probiotic and control groups during the study period

The Plaque Index (PI) was evaluated at baseline, after completion of the 14-day probiotic administration period, and at the end of the follow-up period (Day 28) in order to assess changes in dental biofilm accumulation over time.

At baseline, the mean PI value was 0.97 ± 0.44 in the probiotic group and 1.21 ± 0.48 in the control group. Although the control group presented slightly higher plaque accumulation, the difference between groups was not statistically significant (p = 0.165), indicating a comparable oral hygiene status at study initiation.

Following the intervention period, a substantial reduction in plaque accumulation was observed in both groups. However, participants receiving probiotic supplementation exhibited a greater decrease in PI values compared with those in the control group. At Day 14, the mean PI decreased to 0.42 ± 0.40 in the probiotic group, whereas the control group showed a mean value of 0.78 ± 0.52. This difference reached statistical significance (p = 0.042), suggesting that probiotic administration may have contributed to a more effective reduction in dental biofilm during the active supplementation period (Table 2).

At the final evaluation (Day 28), a slight increase in plaque accumulation was observed in the probiotic group, with the mean PI rising to 0.57 ± 0.36. In contrast, the control group demonstrated a modest reduction, reaching a mean PI value of 0.74 ± 0.56. Despite these changes, PI values in both groups remained lower than those recorded at baseline. The difference between groups at Day 28 was no longer statistically significant (p = 0.330), indicating that the beneficial effect observed immediately after probiotic administration tended to diminish during the post-intervention period.

Table 2. Comparison of Plaque Index (PI) values between the probiotic and control groups during the study period

		Day 0	Day 14	Day 28
Probiotic group	Mean ± SD	0,97± 0,44	0,42± 0,40	0,57± 0,36
	N	15	15	15
Control group	Mean± SD	1,21± 0,48	0,78± 0,52	0,74± 0,56
	N	15	15	15
***p* -value**		0,165	0,042*	0,330

The comparative analysis of PI values throughout the study period is presented in Table 2. As illustrated in Figure 2, both groups demonstrated a downward trend in plaque accumulation over time; however, the reduction was more pronounced in the probiotic group, particularly at the end of the supplementation period. The lowest mean PI value recorded during the study was observed in the probiotic group at Day 14, corresponding to the completion of probiotic administration.

The Gingival Index (GI) was assessed at baseline, after completion of the probiotic supplementation period (Day 14), and at the end of the follow-up period (Day 28) to evaluate changes in gingival health and the degree of gingival inflammation over time.

At baseline, the mean GI values were comparable between the two study groups, with no statistically significant differences observed. The probiotic group presented a mean GI of 0.78 ± 0.38, while the control group exhibited a mean value of 0.74 ± 0.44 (p = 0.791), indicating similar gingival conditions at the beginning of the study(Table 3).

Following the 14-day intervention period, both groups demonstrated an improvement in gingival status, reflected by a reduction in GI values. However, the decrease was substantially greater in participants receiving probiotic supplementation. At Day 14, the mean GI value decreased to 0.33 ± 0.34 in the probiotic group, compared with 0.63 ± 0.32 in the control group. This difference was statistically significant (p = 0.020), suggesting a beneficial effect of probiotic administration on gingival health during the active supplementation period.

Table 3. Comparison of Gingival Index (GI) values between the probiotic and control groups during the study period

		Day 0	Day 14	Day 28
Probiotic group	Mean± SD	0,78± 0,38	0,33± 0,34	0,36± 0,30
	N	15	15	15
Control group	Mean± SD	0,74± 0,44	0,63± 0,32	0,59± 0,26
	N	15	15	15
p -value		0,791	0,020*	0,033*

At the final assessment (Day 28), GI values remained lower than baseline levels in both groups. The probiotic group maintained a lower mean GI value (0.36 ± 0.30) compared with the control group (0.59 ± 0.26). The difference between groups remained statistically significant (p = 0.033), indicating that the beneficial effect observed following probiotic administration persisted throughout the follow-up period.

The comparative analysis of Gingival Index values recorded during the study period is presented in Table 3. As illustrated in Figure 2, both groups exhibited a progressive reduction in gingival inflammation over time; however, the decrease was more pronounced in the probiotic group at both post-baseline evaluations. The lowest GI values were observed among participants receiving probiotic supplementation, supporting the potential role of probiotics in improving gingival health and reducing signs of gingival inflammation.

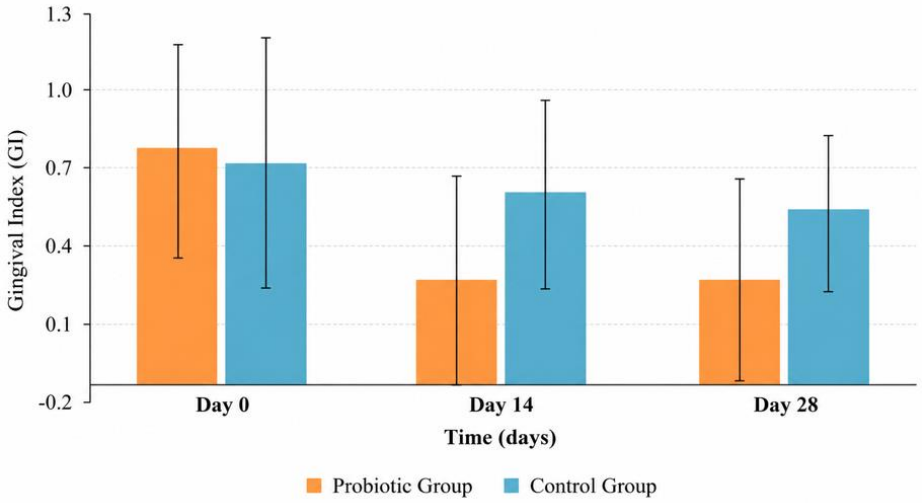

Figure 2. Temporal evolution of Gingival Index (GI) values in the probiotic and control groups during the study period. Error bars represent standard deviations

Table 4. Within-group comparison of Plaque Index (PI), Gingival Index (GI), and salivary pH values in the probiotic group during the study period

	Plaque Index			**Gingival index**			**pH values**		
	N	Mean± SD	*p*	N	Mean± SD	*p*	N	Mean± SD	*p*
Day 0	15	0,97± 0,44	0,003*	15	0,78± 0,38	0,004*	15	6,75± 0,42	0,573
Day14	15	0,42± 0,40		15	0,33± 0,34		15	6,82± 0,21	
Day 0	15	0,97± 0,44	0,017*	15	0,78± 0,38	0,005*	15	6,75± 0,42	0,883
Day 28	15	0,57± 0,36		15	0,36± 0,30		15	6,77± 0,30	
Day 14	15	0,42± 0,40	0,298	15	0,33± 0,34	0,801	15	6,82± 0,21	0,606
Day 28	15	0,57± 0,36		15	0,36± 0,30		15	6,77± 0,3	

DISCUSSIONS

The present study evaluated the effects of a multi-strain oral probiotic formulation on salivary pH, plaque accumulation, and gingival inflammation in young adults. The findings demonstrated that probiotic supplementation was associated with significant improvements in clinical oral health parameters, particularly regarding plaque control and gingival status. Participants receiving probiotics exhibited significantly lower Plaque Index values at the end of the intervention period and significantly lower Gingival Index values at both post-baseline evaluations compared with the control group. In contrast, salivary pH remained relatively stable throughout the study and did not differ significantly between groups. Furthermore, the within-group analysis revealed that the reductions in plaque accumulation and gingival inflammation persisted during the washout period, suggesting that the beneficial effects of probiotic administration may extend beyond the active supplementation phase. These findings support the potential role of probiotics as adjunctive agents in preventive oral healthcare and contribute to the growing body of evidence highlighting the importance of microbiome-modulating strategies for maintaining oral health.

The findings of the present study demonstrated that probiotic supplementation was associated with significant improvements in both plaque accumulation and gingival health. A

significant reduction in Plaque Index values was observed following the 14-day intervention period, while Gingival Index values remained significantly lower than baseline levels even after the washout period. These results are consistent with those reported by Kavitha et al. [10] (2022), who evaluated the effects of probiotic lozenges in children aged 6–12 years and found statistically significant reductions in both plaque and gingival index scores following one month of probiotic administration. In their study, the probiotic group exhibited substantial improvements in oral hygiene and gingival status compared with the placebo group, supporting the potential role of probiotics as adjunctive agents in oral disease prevention. Similar to our findings, the authors suggested that probiotics may contribute to plaque control by inhibiting the growth of pathogenic microorganisms, reducing bacterial adhesion to tooth surfaces, and modulating the composition of the oral biofilm toward a less pathogenic microbial community. Furthermore, the observed reduction in gingival inflammation may be explained by the immunomodulatory properties of probiotic strains, which have been shown to influence local inflammatory responses and promote oral microbial homeostasis. Collectively, these findings reinforce the growing body of evidence supporting the use of probiotics as a complementary strategy for improving oral health and maintaining periodontal stability.

The present findings are also in agreement with those reported by García et al. (2021), [8]who investigated the effects of Lactobacillus reuteri DSM 17938 and ATCC PTA 5289 supplementation on oral health parameters in adolescents. Although the authors observed improvements in plaque, gingival, and bleeding indices, as well as a slight increase in salivary pH, these changes did not reach statistical significance. Nevertheless, the overall trend indicated a favorable effect of probiotic administration on oral health. Similarly, in the present study, probiotic supplementation was associated with lower plaque and gingival index values, while salivary pH remained relatively stable throughout the observation period. The differences observed between the two studies may be partly explained by variations in study design, probiotic strains, participant characteristics, duration of supplementation, and sample size. García et al. emphasized that the absence of statistically significant findings could be related to the limited number of participants included in the trial, highlighting the need for larger randomized clinical studies to better clarify the clinical impact of probiotics on oral health outcomes. Furthermore, the authors reported that the persistence of probiotic strains in the oral cavity appears to be temporary, which may explain why probiotic-associated benefits tend to diminish after discontinuation of supplementation. These observations are consistent with the slight increase in Plaque Index observed during the washout period in our study, despite values remaining below baseline levels.

One of the notable findings of the present study was the absence of significant changes in salivary pH following probiotic supplementation. Although a slight increase in pH values was observed during the intervention period, the differences were not statistically significant. Similar observations have been reported by García et al. (2021), who found a modest increase in salivary pH among adolescents receiving Lactobacillus reuteri supplementation, without reaching statistical significance[8]. A possible explanation for these findings is that participants in both studies exhibited baseline salivary pH values within the physiological range, leaving limited room for measurable improvement. Furthermore, salivary pH is influenced by multiple factors, including diet, salivary flow rate, buffering capacity, and individual oral hygiene practices, which may mask subtle probiotic-related effects. These observations suggest that the beneficial impact of probiotics on oral health may be mediated primarily through modulation of the oral microbiota and inflammatory response rather than through substantial alterations in salivary pH.

The beneficial effects observed in the present study may be explained by several biological mechanisms attributed to probiotic microorganisms. Probiotic strains are capable of

competing with pathogenic bacteria for adhesion sites on oral surfaces, producing antimicrobial substances such as bacteriocins, and modulating the ecological balance of the oral microbiome. In addition, probiotics may influence host immune responses by reducing the production of pro-inflammatory cytokines and promoting a more balanced inflammatory environment within gingival tissues. These mechanisms may contribute to the reduction of dental biofilm accumulation and gingival inflammation observed among participants receiving probiotic supplementation. The persistence of improved Gingival Index values during the washout period may further suggest that probiotics induce temporary ecological and immunological changes that continue to exert beneficial effects even after supplementation has ceased.

Strengths of the present study include the controlled design, the use of validated clinical indices, the inclusion of a washout period, and the simultaneous evaluation of salivary and clinical oral health parameters.

Several limitations of the present study should be acknowledged when interpreting the results. First, the relatively small sample size may have limited the statistical power to detect subtle differences between groups, particularly regarding salivary pH. Second, the duration of probiotic administration and follow-up was relatively short, which restricts the ability to evaluate the long-term effects and sustainability of the observed improvements in oral health parameters. Third, the study relied exclusively on clinical indicators, without incorporating microbiological analyses of the oral biofilm or salivary biomarkers that could have provided additional insight into the mechanisms underlying the observed effects. Furthermore, the study population consisted of healthy young adults with generally good oral health, which may limit the generalizability of the findings to other populations, such as children, older adults, or individuals with active periodontal disease. Despite these limitations, the controlled study design, the inclusion of a washout period, and the simultaneous evaluation of salivary pH, plaque accumulation, and gingival inflammation represent important strengths that contribute to the understanding of the potential role of probiotics in preventive oral healthcare.

From a clinical perspective, the results of the present study support the potential incorporation of probiotics into preventive oral healthcare strategies. Although probiotics cannot replace conventional oral hygiene measures such as tooth brushing and professional plaque control, they may represent a valuable adjunctive approach for maintaining oral health. The observed improvements in plaque accumulation and gingival status suggest that probiotic supplementation may contribute to creating a more favorable oral environment and reducing early signs of periodontal inflammation. Such benefits may be particularly relevant for individuals at increased risk of plaque accumulation or gingival disease, as well as for populations seeking non-pharmacological strategies to support oral health maintenance.

CONCLUSIONS

The findings of the present study suggest that short-term supplementation with a multi-strain chewable probiotic formulation may contribute to improvements in oral health among young adults. Probiotic administration was associated with significant reductions in both Plaque Index and Gingival Index values, indicating beneficial effects on dental biofilm control and gingival health. Notably, the improvement in gingival status persisted throughout the washout period, suggesting that the effects of probiotic supplementation may extend beyond the active intervention phase.

In contrast, salivary pH remained within the physiological range throughout the study and was not significantly influenced by probiotic administration. These findings indicate that the beneficial effects of probiotics may be mediated primarily through modulation of the oral

microbiota and host inflammatory responses rather than through substantial alterations in salivary physicochemical characteristics.

Overall, the results support the potential use of probiotics as adjunctive agents in preventive oral healthcare and highlight their role as microbiome-based strategies for maintaining oral health. Further randomized clinical trials with larger sample sizes, longer follow-up periods, and microbiological assessments are warranted to confirm these findings and better elucidate the mechanisms underlying the observed clinical benefits.

Conflicts of Interest

The authors declare no conflict of interest.

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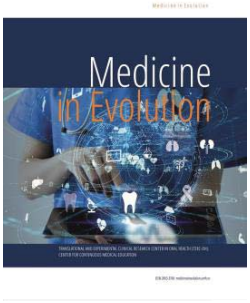
Can Bioactivity Preserve Esthetics? Color Stability of Resin Composites Following Exposure to Everyday Beverages



https://doi.org/10.70921/medev.v32i2.2037

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Received: 09 June 2026; Accepted: 15 June 2026; Published: 30 June 2026

Abstract

1.Background/Objectives: Resin-based composites are widely used restorative materials; however, their long-term esthetic stability may be affected by exposure to commonly consumed beverages. This in vitro study aimed to evaluate and compare the color stability of a bioactive resin composite (Predicta® Bioactive Bulk Fill) and a conventional microhybrid resin composite (Filtek™ Z250 Universal Restorative) following immersion in coffee, Coca-Cola, and Nestea. 2.Methods: Forty disc-shaped specimens (n = 40) were fabricated, polished, and randomly allocated to four immersion media (distilled water, coffee, Coca-Cola, and Nestea; n = 5/group). After seven days of immersion at 37°C, color measurements were performed using a VITA Easyshade V spectrophotometer according to the CIE Lab* color system. Descriptive statistics and two-way ANOVA were applied, with statistical significance established at $p < 0.05$. 3.Results: Coffee produced the greatest color alterations in both materials, resulting in the highest increases in b* values and estimated color differences. Predicta Bioactive showed consistently greater discoloration than Filtek Z250 in all immersion media. The highest estimated ΔE*ab values were recorded after coffee immersion (15.77 for Predicta Bioactive and 7.48 for Filtek Z250), followed by Nestea and Coca-Cola. 4.Conclusion: Beverage exposure significantly influenced color stability, with Predicta Bioactive showing greater discoloration than Filtek Z250 and coffee exhibiting the highest staining potential.

Keywords: Dental Resin Composites; Bioactive Materials; Color Stability; Beverage Consumption; Surface Properties; Aging; In Vitro Techniques

INTRODUCTION

Resin-based composites remain the most widely used direct restorative materials in contemporary dentistry due to their favorable esthetic properties, adhesive bonding capability, and continuous improvements in mechanical performance. Recent advances in restorative biomaterials have expanded the concept of dental composites beyond passive defect replacement, leading to the development of bioactive restorative materials designed to interact dynamically with the oral environment through ion release, remineralization potential, and antimicrobial activity. These materials aim not only to restore lost tooth structure but also to contribute to the prevention of secondary caries and the maintenance of oral health. Recent reviews have highlighted the growing interest in bioactive resin composites as a promising generation of restorative materials capable of combining functional, biological, and esthetic benefits [1–3].

Bioactive resin composites typically incorporate bioactive glass, calcium phosphate compounds, fluoride-releasing fillers, or other ion-releasing particles that promote remineralization and enhance the biological performance of restorations. Their ability to respond to changes in the oral environment has led to their classification among "smart" dental materials. Nevertheless, despite these advantages, concerns remain regarding the long-term stability of their physical and esthetic properties, particularly when exposed to the complex chemical challenges encountered in everyday life [4–6].

Color stability is one of the most important determinants of the clinical success of esthetic restorations. Patients increasingly expect restorations to maintain their original appearance over time, especially in visible areas of the dentition. However, discoloration remains a common reason for restoration replacement. Color changes may result from intrinsic factors, such as resin matrix degradation and water sorption, as well as extrinsic factors including exposure to dietary pigments and acidic beverages. The extent of discoloration depends on material composition, filler characteristics, degree of conversion, surface properties, and environmental conditions [7,8].

Modern dietary habits expose restorative materials to a wide range of staining and erosive agents. Coffee is recognized as one of the most chromogenic beverages due to its high concentration of yellow-brown pigments and tannins, while carbonated soft drinks such as Coca-Cola may promote surface degradation through their acidic pH. Ready-to-drink iced teas, including Nestea®, combine both acidic and chromogenic characteristics, potentially affecting the optical performance of resin-based restorations. Several studies have demonstrated that prolonged exposure to these beverages can significantly alter the color and surface characteristics of composite materials [9–12].

The optical behavior of resin composites is strongly influenced by their chemical composition and microstructure. The organic matrix, filler content, filler size distribution, refractive index matching, and water sorption characteristics all contribute to the overall color stability of restorative materials. Exposure to aqueous environments may promote water uptake and hydrolytic degradation of the resin matrix, facilitating the penetration and adsorption of exogenous pigments. Consequently, materials exhibiting increased hydrophilicity or greater water sorption may be more susceptible to clinically perceptible discoloration over time [8,13].

Bioactive restorative materials introduce additional complexity because their therapeutic mechanisms often rely on ion exchange and interaction with the surrounding oral environment. While these features may provide biological benefits such as remineralization and pH buffering, they may also influence surface integrity, water sorption, and optical stability. Long-term ion release and exposure to aqueous media have been associated with

alterations in the physicochemical properties of certain bioactive materials, raising questions regarding their ability to maintain esthetic performance under everyday dietary challenges [3,14].

From a clinical perspective, color alterations exceeding perceptibility or acceptability thresholds may compromise patient satisfaction and lead to restoration replacement despite the absence of structural failure. Therefore, evaluating the color stability of emerging bioactive composites is essential not only for understanding their material behavior but also for assessing their long-term clinical suitability in esthetically demanding situations. As the use of bioactive restorative materials continues to expand, evidence regarding their resistance to staining and discoloration becomes increasingly relevant for evidence-based material selection in daily dental practice [15,16].

Although numerous investigations have evaluated the staining susceptibility of conventional resin composites, evidence regarding the esthetic behavior of bioactive restorative composites remains limited and sometimes contradictory. The ion-releasing nature and increased water interaction associated with some bioactive materials may influence their resistance to discoloration. Consequently, direct comparisons between bioactive and conventional composites under identical staining conditions are necessary to better understand their long-term esthetic performance and clinical applicability [14,15].

Aim and objectives

The aim of this in vitro study was to evaluate and compare the color stability of a bioactive restorative composite (Predicta® Bioactive Bulk Fill, Parkell Inc., Brentwood, New York, USA) and a conventional resin-based composite (Filtek™ Z250 Universal Restorative, 3M Oral Care, St. Paul, Minnesota, USA) after immersion in commonly consumed beverages. Given the increasing clinical use of bioactive restorative materials and the growing demand for long-term esthetic performance, understanding their behavior under staining conditions is of particular clinical relevance.

The specific objectives of the study were to assess the color changes exhibited by both restorative materials after immersion in coffee, Coca-Cola, and Nestea; to compare the staining susceptibility of the bioactive and conventional composites; to evaluate the influence of different beverages on the esthetic behavior of the investigated materials; and to determine whether the bioactive composite demonstrated superior color stability compared with the conventional composite under identical experimental conditions.

The null hypothesis tested was that neither the restorative material nor the immersion medium would significantly influence the color stability of the investigated resin composites after seven days of exposure.

MATERIAL AND METHODS

This in vitro study evaluated the color stability of two commercially available resin-based restorative materials: a bioactive resin composite, Predicta® Bioactive Bulk Fill (Parkell Inc., Brentwood, NY, USA), and a conventional microhybrid resin composite, Filtek™ Z250 Universal Restorative (3M Oral Care, St. Paul, MN, USA). Both materials were used in shade A2 to eliminate the influence of shade variability on color measurements. Predicta Bioactive was selected because of its ion-releasing and remineralizing potential, while Filtek Z250 was chosen as a well-established conventional composite with documented clinical performance and color stability. The main characteristics of the investigated materials are presented in Table 1.

Table 1. Characteristics of the investigated restorative materials

Material	Manufacturer	Shade	Composite Type	Resin Matrix	Filler Content
Predicta® Bioactive Bulk Fill	Parkell Inc., Brentwood, NY, USA	A2	Bioactive composite	Modified urethane dimethacrylate resin matrix with ionic resin components	Bioactive glass, calcium phosphate and silica fillers (~74 wt%)
Filtek™ Z250 Universal Restorative	3M Oral Care, St. Paul, MN, USA	A2	Microhybrid composite	Bis-GMA, UDMA, Bis-EMA	Zirconia/Silica fillers (~82 wt%)

A total of forty disc-shaped specimens were fabricated, with twenty specimens prepared from each restorative material. The sample size was determined based on the experimental design, which included two restorative materials and four immersion media (distilled water, coffee, Coca-Cola, and Nestea), resulting in eight experimental groups. Five specimens were allocated to each group (n = 5), yielding a total of forty specimens (2 materials × 4 immersion conditions × 5 specimens). This sample size is consistent with previous in vitro studies investigating the color stability of resin-based restorative materials following exposure to staining beverages and was considered sufficient to detect clinically relevant differences among groups while maintaining experimental feasibility. The selected sample size was also in accordance with similar in vitro investigations[11] evaluating discoloration and optical changes in dental resin composites, where group sizes ranging from five to ten specimens are commonly employed.

Standardized cylindrical plexiglass molds measuring 10 mm in diameter and 2 mm in thickness were used for specimen preparation. The composite material was inserted into the mold in a single increment and covered with a Mylar strip to obtain a smooth surface and minimize the oxygen-inhibited layer. A glass slide was gently pressed over the strip to remove excess material and standardize specimen thickness.

Polymerization was performed using a LED curing unit (Bluephase G2, Ivoclar Vivadent, Schaan, Liechtenstein) operating at an intensity of 1200 mW/cm². Each specimen was light-cured for 40 s on both sides, with the curing tip positioned approximately 1 mm from the specimen surface. The output intensity of the curing unit was periodically verified using a calibrated radiometer according to the manufacturer's recommendations.

After polymerization, all specimens were removed from the molds and visually inspected to exclude samples presenting voids, cracks, or surface defects.

To standardize surface conditions prior to color assessment, all specimens were polished using a multi-step polishing system (Sof-Lex™, 3M ESPE Dental Products, St. Paul, MN, USA) under low-speed rotation. Polishing was performed sequentially using coarse, medium, fine, and superfine discs for approximately 20 s per step. Following polishing, specimens were ultrasonically cleaned in distilled water for 5 min to remove polishing residues and subsequently stored in distilled water at 37°C for 24 h before baseline color measurements.

The specimens were randomly assigned to four experimental groups according to the immersion medium:

- Distilled water (control)
- Coffee
- Coca-Cola
- Nestea®

For each restorative material, five specimens were included in each experimental group (n = 5).

Coffee solution was prepared daily by dissolving 5 g of instant coffee in 150 mL of boiling distilled water. Coca-Cola® (The Coca-Cola Company, Atlanta, GA, USA) and

Nestea® iced tea were used directly from newly opened containers to ensure chemical consistency throughout the experiment.

All immersion solutions were maintained at 37°C to simulate oral conditions. Specimens were individually immersed in sealed containers containing 20 mL of the corresponding solution and stored continuously for 7 consecutive days.

To minimize variations in chemical composition and staining capacity, all solutions were renewed every 24 h. Control specimens were stored in distilled water at 37°C throughout the entire experimental period.

Color evaluation was performed using a digital spectrophotometer (VITA Easyshade V, VITA Zahnfabrik, Bad Säckingen, Germany) according to the Commission Internationale de l'Éclairage (CIE) L*a*b* color system. Measurements were obtained after specimen preparation and following seven days of immersion in the experimental media. For each specimen, three consecutive measurements were recorded and averaged to minimize measurement variability.

The color characteristics of the investigated materials were assessed using the three-dimensional CIELAB color space, where L* represents lightness, a* indicates the red–green chromatic axis, and b* corresponds to the yellow–blue chromatic axis (Figure 1). Changes in these coordinates were used to evaluate the influence of the immersion media on the optical behavior of the tested restorative materials. Particular attention was given to variations in the b* coordinate, as increases in positive b* values are commonly associated with clinically perceptible yellow discoloration induced by staining beverages.

$$\Delta E*ab = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2}$$

Figure 1. Schematic representation of the CIE L*a*b* color space

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics software (Version 30.0, IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean values and standard deviations, were calculated for all investigated parameters. Data distribution was assessed using the Shapiro–Wilk test.

Differences in the CIELAB color coordinates (L*, a*, and b*) among the experimental groups were analyzed using two-way analysis of variance (Two-Way ANOVA), considering restorative material and immersion medium as independent factors. When significant differences were identified, Tukey's post hoc test was applied for pairwise comparisons. Statistical significance was established at $p < 0.05$.

Scheme I: Experimental workflow of specimen preparation, immersion protocol, color evaluation, and statistical analysis

RESULTS

Color Coordinate Changes Following Beverage Exposure

The mean color coordinate values obtained after seven days of immersion are presented in Table 2. Distinct differences were observed between the tested restorative materials and immersion media, indicating that both factors influenced the optical behavior of the investigated composites.

Table 1. Mean b* values of the investigated materials after seven days of immersion

Material	Control	Coca-Cola	Coffee	Nestea
Predicta Bioactive	23.39 ± 2.18	32.86 ± 0.47	37.59 ± 2.08	33.21 ± 0.90
Filtek Z250	21.63 ± 1.12	21.13 ± 0.64	26.09 ± 0.36	22.95 ± 0.62

Coffee produced the highest increase in the yellow-blue coordinate (b*) for both materials. The greatest mean value was recorded for Predicta Bioactive specimens immersed in coffee (37.59 ± 2.08), followed by Nestea (33.21 ± 0.90) and Coca-Cola (32.86 ± 0.47). In contrast, Filtek Z250 demonstrated substantially lower b* values under identical immersion conditions, with mean values of 26.09 ± 0.36, 22.95 ± 0.62, and 21.13 ± 0.64 for coffee, Nestea, and Coca-Cola, respectively.

Figure 2 illustrates the mean b values recorded after seven days of immersion. The graphical representation highlights the greater susceptibility of Predicta Bioactive to yellow discoloration and confirms the stronger staining effect of coffee compared with Coca-Cola and Nestea.

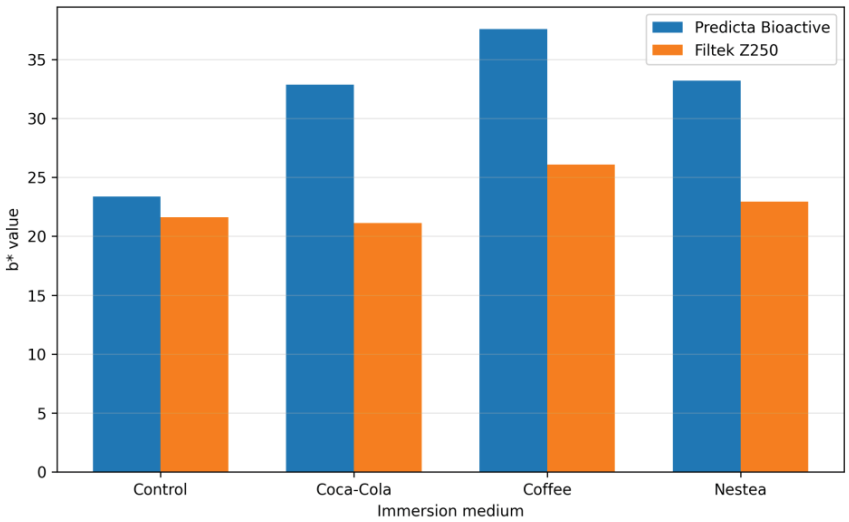

Figure 2. Mean b* values of Predicta Bioactive and Filtek Z250 after seven days of immersion

Compared with the control group, Predicta Bioactive exhibited marked increases in b* values after immersion in all tested beverages, particularly coffee (+14.20 units), followed by Nestea (+9.82 units) and Coca-Cola (+9.47 units). Conversely, Filtek Z250 showed more limited color alterations, with the largest increase observed after coffee exposure (+4.46 units), followed by Nestea (+1.32 units), while Coca-Cola produced virtually no increase in yellowness (−0.50 units relative to control).

Changes in Lightness (L)*

The mean L* values demonstrated a reduction in lightness after immersion, particularly in the coffee groups. Predicta Bioactive specimens showed a decrease from a mean control value of 82.0 to 76.45 following coffee immersion, while Filtek Z250 decreased from 80.59 to 74.90. These findings indicate a darkening effect induced by coffee for both restorative materials. The Coca-Cola and Nestea groups exhibited less pronounced reductions in lightness. For Predicta Bioactive, mean L* values were 82.86 after Coca-Cola exposure and 80.98 after Nestea exposure. Filtek Z250 showed mean values of 79.58 and 80.84 after Coca-Cola and Nestea immersion, respectively.

Changes in the Red-Green Coordinate (a)*

Differences were also observed in the a* coordinate. Predicta Bioactive exhibited a shift toward more positive a* values following immersion, particularly in the coffee group (mean a* = 2.36), indicating increased red chromaticity. The corresponding mean value for Filtek Z250 after coffee immersion was 0.44. Similarly, Coca-Cola and Nestea induced greater variations in a* values for Predicta Bioactive compared with Filtek Z250. These findings suggest that the bioactive material experienced more pronounced chromatic alterations in response to beverage exposure.

Comparative Analysis between Materials

Direct comparison of the two restorative materials revealed consistently higher discoloration-related color coordinate values for Predicta Bioactive. The differences between materials were particularly evident after immersion in coffee and Nestea. Mean b* values were approximately 11.5 units higher for Predicta Bioactive than for Filtek Z250 after coffee immersion and 10.3 units higher after Nestea immersion. Following Coca-Cola exposure, the difference remained substantial at approximately 11.7 units.

Among the tested beverages, coffee exhibited the highest staining potential for both restorative materials. For Predicta Bioactive, coffee induced the most pronounced color

changes, followed by Nestea and Coca-Cola, while the control group showed the lowest degree of discoloration. A similar trend was observed for Filtek Z250, with coffee producing the greatest color alteration, followed by Nestea, whereas the control and Coca-Cola groups demonstrated comparable and substantially lower color changes. These findings indicate that the bioactive composite was more susceptible to beverage-induced color changes than the conventional microhybrid composite under the conditions of the present study.

Visual Assessment

Representative photographs of the specimens after seven days of immersion are presented in Figure 3. Visual examination corroborated the instrumental color measurements, revealing more pronounced discoloration in the coffee-exposed groups and greater color alteration for Predicta Bioactive compared with Filtek Z250. The control specimens maintained the most stable appearance, while Coca-Cola and Nestea produced intermediate staining effects.

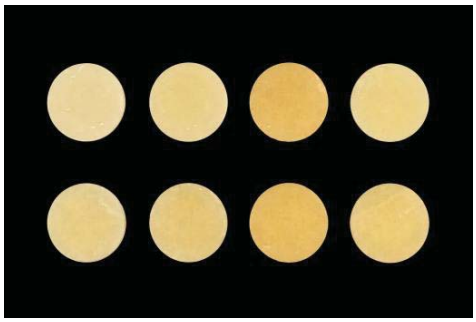

Figure 3. Representative images of the tested specimens after seven days of immersion.
The upper row corresponds to Predicta® Bioactive Bulk Fill (Parkell Inc., Brentwood, NY, USA), whereas the lower row corresponds to Filtek™ Z250 Universal Restorative (3M Oral Care, St. Paul, MN, USA). From left to right, the specimens represent the following experimental conditions: control (distilled water), Coca-Cola®, coffee, and Nestea®.

Color Difference Analysis

To provide an overall assessment of discoloration, color differences were estimated using the mean L*, a*, and b* values obtained for each experimental group relative to the corresponding control group. The highest color difference was observed in the coffee groups for both restorative materials. Predicta Bioactive exhibited an estimated ΔE value of 15.77 after coffee immersion, followed by Nestea (10.09) and Coca-Cola (9.55). In contrast, Filtek™ Z250 demonstrated lower color differences, with estimated ΔE values of 7.48, 2.86, and 3.43 following immersion in coffee, Nestea, and Coca-Cola, respectively (Table 3).

The bioactive composite consistently exhibited greater color alteration than the conventional composite under all immersion conditions. The largest difference between materials was observed after coffee exposure, where Predicta Bioactive showed approximately twice the color change recorded for Filtek™ Z250. Similar trends were observed for Coca-Cola and Nestea immersion.

These findings indicate that beverage composition significantly influenced the optical behavior of both restorative materials, with coffee producing the most pronounced discoloration. Furthermore, the bioactive restorative material appeared more susceptible to beverage-induced color changes than the conventional microhybrid composite under the experimental conditions of the present study.

Table 3. Estimated color difference (ΔE*ab) relative to control specimens after seven days of immersion

Material	Coca-Cola	Coffee	Nestea
Predicta Bioactive	9.55	15.77	10.09
Filtek™ Z250	3.43	7.48	2.86

Figure 4 provides a graphical representation of the estimated ΔE*ab values obtained after seven days of immersion. The highest color differences were observed in the coffee groups, while Predicta Bioactive consistently showed greater discoloration than Filtek™ Z250 regardless of the immersion medium.

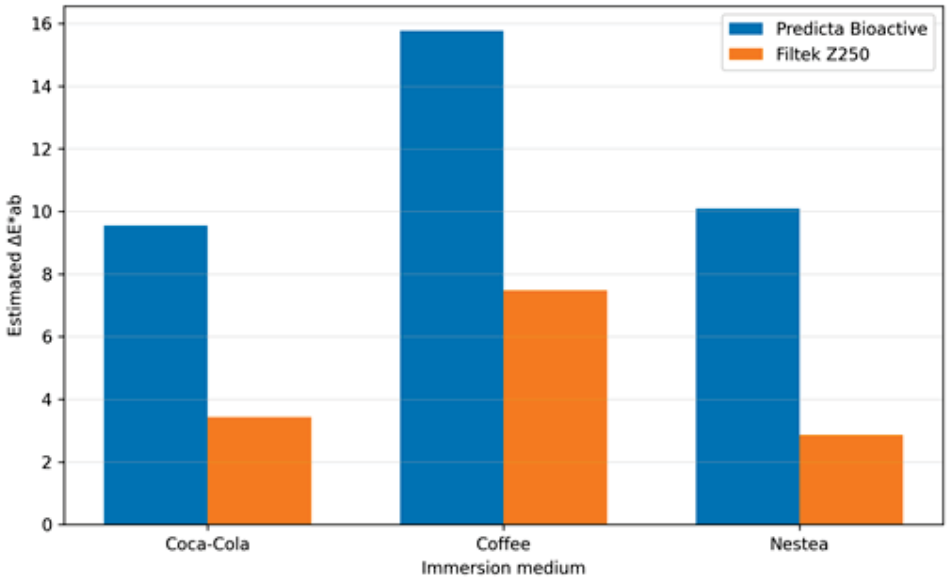

Figure 4. Estimated ΔE*ab values relative to the control group after seven days of immersion

DISCUSSIONS

The present in vitro study evaluated the esthetic behavior of a bioactive resin composite, Predicta® Bioactive Bulk Fill, compared with a conventional microhybrid composite, Filtek™ Z250 Universal Restorative, after seven days of immersion in commonly consumed beverages. The results showed that both the restorative material and the immersion medium influenced the CIELAB color coordinates and the estimated color difference values. Therefore, the null hypothesis was rejected.

The main finding of this study was that Predicta Bioactive exhibited greater beverage-induced discoloration than Filtek Z250 under all tested conditions. This was particularly evident for the b* coordinate, which increased markedly in the Predicta groups exposed to coffee, Nestea, and Coca-Cola. Since positive b* values indicate a shift toward yellow, these findings suggest that the bioactive composite was more susceptible to yellow discoloration than the conventional microhybrid composite. This behavior may be related to the specific composition and functional mechanism of bioactive restorative materials. Bioactive resin-based materials are designed to interact with the surrounding oral environment through ion release, remineralizing activity, and water-mediated processes; however, these same mechanisms may increase water sorption, solubility, and pigment penetration, thereby affecting optical stability. Recent reviews on bioactive dental resins have emphasized that, although these materials have important preventive and remineralizing potential, their long-term physicochemical and clinical stability remains a key challenge[1,3].

Coffee produced the most pronounced color alteration in both restorative materials. This result is consistent with several recent in vitro studies reporting coffee as one of the most aggressive staining media for resin composites. Korać et al.[17] found that coffee and tea produced color changes above the clinical acceptability threshold in tested resin composites, while a 2024 study also reported that coffee and red wine caused greater composite discoloration than tea and cola[18]. The high staining potential of coffee may be explained by its yellow-brown chromogens, tannins, and polyphenolic compounds, which may be adsorbed onto the composite surface or absorbed into the resin matrix. In the present study, coffee generated the highest estimated ΔE*ab values for both Predicta Bioactive and Filtek Z250, confirming its strong chromogenic effect.

The present findings are also in agreement with the study by Uctasli et al.[19], who evaluated the color stability of six resin composites after exposure to commonly consumed beverages and showed that staining susceptibility depends on both the material and the type of beverage. Similarly, recent investigations on resin-based materials exposed to different beverages have shown that coffee and tea-based drinks produce more pronounced discoloration than cola-type beverages[20]. In our study, coffee caused the highest discoloration, while Nestea produced intermediate changes and Coca-Cola showed a lower staining effect, especially for Filtek Z250. This confirms that beverages with both chromogenic compounds and acidic properties may have a stronger impact on color stability than acidic beverages with limited pigment content.

Nestea also produced visible and instrumental color changes, particularly in Predicta Bioactive. Although its staining effect was lower than that of coffee, the increased b* values observed after Nestea immersion indicate a tendency toward yellow discoloration. This may be related to its tea-derived pigments, colorants, sugars, and acidic components. Previous studies and systematic reviews have shown that tea and tea-based beverages can significantly affect the color stability of tooth-colored restorative materials[17,21]. From this perspective, the findings of the present study support the idea that iced tea beverages should not be considered neutral from an esthetic standpoint, especially in patients with frequent consumption patterns.

Coca-Cola showed different effects depending on the restorative material. In Filtek Z250, Coca-Cola produced minimal changes in b* values compared with the control group, suggesting relatively good color stability in this acidic but less chromogenic medium. In contrast, Predicta Bioactive showed a marked increase in b* values after Coca-Cola immersion. This finding suggests that, in the case of the bioactive composite, the acidic environment may have promoted surface or matrix alterations that increased susceptibility to discoloration, even without strong pigment exposure. Acidic beverages may soften the resin matrix, affect the filler–matrix interface, and increase surface roughness, thereby facilitating subsequent optical changes. Recent studies evaluating acidic beverages have reported that these media can alter the microhardness, surface characteristics, and optical properties of resin composite materials[22,23].

The greater discoloration observed for Predicta Bioactive may also be discussed in relation to the balance between bioactivity and esthetic stability. The clinical advantage of bioactive composites is generally associated with ion release, remineralization, and possible pH-buffering effects. However, these properties often require interaction with water and the surrounding environment. Long-term immersion studies on bioactive glass-containing composites have shown that water sorption, solubility, and porosity are relevant parameters when assessing the behavior of bioactive materials over time[14]. Therefore, the higher discoloration found in Predicta Bioactive may reflect a trade-off between functional bioactivity and resistance to staining. This does not diminish the biological potential of

bioactive materials, but it highlights the importance of evaluating their esthetic performance under clinically relevant dietary conditions.

Filtek Z250 showed better color stability than Predicta Bioactive in all immersion media. This may be associated with its conventional resin matrix and zirconia/silica filler system, which may provide a more stable optical structure and lower interaction with aqueous media. Although conventional composites are not bioactive, their long-standing clinical use and optimized filler–matrix structure may contribute to predictable esthetic performance. Recent literature continues to show that resin composite staining is strongly influenced by material composition, resin matrix hydrophilicity, filler loading, particle size, and polishing quality[19,24]. Thus, the lower discoloration of Filtek Z250 in the present study may be attributed to its more stable conventional formulation.

The estimated ΔEab values confirmed the trends observed in the individual CIELAB coordinates. Predicta Bioactive showed higher estimated color difference values than Filtek Z250 after immersion in all tested beverages. The highest estimated ΔEab was recorded after coffee immersion, followed by Nestea and Coca-Cola. Although these values were calculated using group mean coordinates rather than individual baseline–post-immersion measurements, they provide an overall indication of the magnitude of color alteration. Importantly, all estimated ΔE*ab values for Predicta Bioactive were above commonly used clinical acceptability thresholds reported in dental color research, suggesting that the observed changes would likely be clinically relevant. However, this interpretation should be made cautiously because true ΔE values should ideally be calculated for each specimen using paired baseline and final measurements.

From a clinical perspective, the results are relevant because esthetic failure remains an important reason for restoration replacement, even when mechanical integrity is preserved. Patients receiving restorations in visible areas may perceive color mismatch or progressive discoloration as unacceptable. The present findings suggest that dietary habits, particularly frequent coffee or iced tea consumption, should be considered when selecting restorative materials for esthetically demanding regions. Bioactive resin composites may be advantageous in patients with high caries risk because of their potential remineralizing and ion-releasing behavior, but clinicians should be aware that this benefit may not automatically translate into superior color stability. Recent systematic reviews on bioactive resin materials indicate that their clinical superiority over conventional composites is not yet consistently demonstrated, especially when long-term outcomes are considered[15,25].

Therefore, material selection should be individualized. In posterior areas or in patients with increased caries risk, a bioactive restorative material may offer biological advantages. However, in anterior or highly visible areas, where long-term color matching is critical, conventional composites with proven esthetic stability may remain preferable unless further evidence confirms improved optical performance of bioactive alternatives. Preventive dietary counseling is also important. Patients with frequent consumption of coffee, tea-based drinks, or acidic beverages should be informed about the potential risk of restoration discoloration and the need for regular maintenance, polishing, and recall visits.

A strength of the present study is the direct comparison between a commercially available bioactive composite and a well-established conventional microhybrid composite under identical experimental conditions. Another strength is the use of commonly consumed beverages that reflect realistic dietary exposures. The standardized specimen dimensions, shade selection, polishing protocol, immersion temperature, solution renewal, and instrumental color measurement using the CIELAB system also enhance the reproducibility of the study. Moreover, the inclusion of both individual CIELAB coordinates and estimated ΔE*ab values provides a broader understanding of the optical changes induced by each beverage.

Nevertheless, several limitations should be acknowledged. First, the study was conducted in vitro and cannot fully reproduce the complexity of the oral environment, where saliva, acquired pellicle, oral microbiota, brushing, thermal cycling, pH fluctuations, and masticatory forces influence staining and material degradation. Second, the immersion protocol involved continuous exposure for seven days, which represents an accelerated staining model rather than a direct simulation of normal beverage consumption. Third, the study evaluated only two restorative materials and three beverages, which limits generalizability. Fourth, the study focused primarily on optical parameters; additional surface analyses such as roughness, microhardness, scanning electron microscopy, water sorption, and spectroscopic evaluation would help clarify the mechanisms responsible for discoloration. Finally, the estimated ΔEab values were calculated from group mean coordinates; future studies should calculate ΔEab or preferably CIEDE2000 ΔE00 for each specimen using paired baseline and post-immersion values.

Future research should include a larger number of bioactive and conventional composites with different resin matrices, filler systems, and ion-releasing technologies. Longer aging protocols, thermocycling, brushing simulation, artificial saliva storage, and pH-cycling models would provide a more clinically relevant assessment of long-term color stability. It would also be valuable to correlate color changes with surface roughness, water sorption, solubility, microhardness, and ion release. Such multimodal analyses could clarify whether increased discoloration is primarily caused by pigment adsorption, resin matrix degradation, filler–matrix breakdown, or bioactive ion-exchange processes. Clinical studies are also needed to determine whether the in vitro staining susceptibility observed in bioactive composites translates into visible discoloration and restoration replacement in patients.

Within the limitations of the present study, the results indicate that Predicta Bioactive was more susceptible to beverage-induced discoloration than Filtek Z250. Coffee was the most aggressive staining medium for both materials, followed by Nestea, while Coca-Cola showed a lower staining effect, particularly for the conventional composite. These findings suggest that bioactivity does not necessarily preserve esthetic stability and that the clinical use of bioactive composites should consider both their biological benefits and their optical behavior under everyday dietary challenges.

CONCLUSIONS

Within the limitations of this in vitro study, both the restorative material and the immersion medium influenced the color stability of the investigated composites. Predicta® Bioactive Bulk Fill demonstrated greater susceptibility to discoloration than Filtek™ Z250 Universal Restorative following exposure to all tested beverages. Among the immersion media, coffee produced the most pronounced color changes, followed by Nestea, whereas Coca-Cola exhibited the lowest staining potential. The bioactive composite consistently showed greater alterations in the evaluated color parameters, indicating reduced resistance to beverage-induced discoloration compared with the conventional microhybrid composite. These findings suggest that the bioactive properties of restorative materials do not necessarily ensure superior esthetic stability. Consequently, both material characteristics and patients' dietary habits should be considered when selecting restorative materials, particularly in esthetically demanding clinical situations. While bioactive composites offer potential therapeutic benefits, clinicians should carefully balance these advantages against their possible susceptibility to discoloration when planning restorations in visible areas.

Conflicts of Interest

The authors declare no conflict of interest.

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