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PROBLEMS OF EVALUATING THE POTENTIAL PREBIOTIC ACTIVITY OF FOOD AND FEED PRODUCTS

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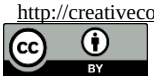

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Abstract. The advancement of scientific research in the development of novel functional food and feed products has led to a significant expansion of their range. Selecting effective ingredients for such products and determining the impact of subsequent processing technologies require assessing their level of functionality, which involves substantial investments of time and material resources. Therefore, the application of methods for evaluating the potential prebiotic properties of food and feed raw materials, as well as ready-to-consume products, is of great interest to both research and industrial laboratories. In 2003, R. Palframan, G.R. Gibson, and R.A. Rastall proposed a method for determining the prebiotic index (PI). This method involves calculating the ratios of cultivated bacteria of the genera Lactobacillus and Bifidobacterium, as well as bacteroides of the family Bacteroidaceae and bacteria of the genus Clostridium, grown on the same nutrient medium, to the total number of microorganisms grown on that same medium. This paper attempts to improve the aforementioned method for determining the prebiotic index by separately calculating the ratios of cultivated bacteria of the genera Lactobacillus and Bifidobacterium to the bacteria of the same genera grown on identical control nutrient media. Evaluating prebiotic indexes separately allows for a clearer differentiation of the raw material's functional effects compared to the integral prebiotic index, which averages out prebiotic characteristics, thereby complicating the targeted design of products for specific dysbiotic conditions and the needs of human and animal organisms. The determination of the prebiotic index based on the growth of Bifidobacterium and Lactobacillus bacteria upon adding samples of common vegetables to control nutrient media confirmed high selectivity regarding the growth stimulation of individual microbial genera. None of the tested products simultaneously exhibited maximum prebiotic index values for both lacto- and bifidobacteria, which experimentally confirms the validity of the developed approach for the separate calculation of indexes ($BI_{lac.}$ and $BI_{bif.}$).

Keywords: functional food and feed products, prebiotic properties, prebiotic index, bifidobacteria and lactobacilli, vegetables, pomace.

Introduction. Formulation of the problem

The volume of production and consumption of functional food and feed products is steadily increasing. This trend is driven not only by the potential to improve human and animal health but also by the economic benefits of marketing next-generation products. This growth is further propelled by consumer demand for preventative health solutions, rising healthcare costs, and increasing awareness of diet-related diseases [1]. The global functional food market is estimated at USD 395 to 437 billion, with projections forecasting explosive growth to USD 793 billion by 2032 and USD 983 billion by 2034. This expansion is fueled by preventative healthcare practices and post-pandemic dietary shifts, driving rapid consumption growth within the cardiovascular, digestive, and sports nutrition segments [2,3,4].

The benefits of functional feeds within the healthy food supply chain drive the expansion of scientific research and ensure rapid growth in functional feed production volumes. This market is projected to nearly

double by 2034, rising from USD 25.7 billion to USD 53.8 billion [5]. A particularly high demand for these feeds is observed in the rearing of young livestock and poultry, while successful aquaculture farming is virtually impossible without functional feeds [6].

Over the past 10–15 years, the advancement of scientific research in developing novel functional food and feed products has led to a significant expansion of both the product range and the raw materials used for their production. To successfully address the challenges of ingredient selection, as well as the implementation of efficient manufacturing processes and production technologies for functional food and feed products, a robust methodological framework is required to determine the level of their functionality.

Today, the assessment of prebiotic properties for both raw materials and ready-to-consume functional food and feed products has evolved into a complex, multidisciplinary system. This comprehensive process relies heavily on laboratory, microbiological, and biochemical studies. Specifically, evaluating the prebiotic characteristics of food and feed products involves laboratory testing to confirm that the ingredients responsible for the prebiotic effect are resistant to gastric juice, fermentable by microbiota, and capable of selectively stimulating the growth of beneficial bacteria, ultimately leading to the normalization of bodily functions or a distinct therapeutic effect [7]. To obtain a reliable assessment of the prebiotic potential of specific components or products, in vitro studies are typically supplemented by in vivo testing [8]. The key in vitro assessment methods include evaluating prebiotic properties based on their resistance to acidic environments and enzymes at low pH (pH 2–3) and exposure to pepsin, which simulates passage through the stomach and small intestine [9]. Another method is fermentation testing, which assesses the capacity of beneficial microorganisms (Lactobacillus and Bifidobacterium) to utilize the substrate as a carbon source [10]. Additionally, evaluating the production capacity of short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which improve the condition of the intestinal epithelium and the overall microbiome, is also employed [11]. Such an extensive evaluation system for prebiotic characteristics significantly complicates research, resulting in substantial investments of time and resources.

Analysis of recent research and publications

Modern research on the human and animal microbiome indicates that the level of functionality and prebiotic properties of food and feed products can be assessed based on the growth rates of beneficial bacteria, such as Lactobacillus and Bifidobacterium, as key microbial indicators. This is because their growth requires non-digestible carbohydrates (prebiotics) for energy, and their growth rates and metabolic activity serve as standard, quantifiable metrics used by scientists to evaluate prebiotic efficacy [12,13,14,15,16].

However, comparative analysis of growth assessments for bacteria of the genera Lactobacillus and Bifidobacterium is often hindered by the application of different approaches to measuring colony-forming units (CFU). The research process involves serial dilutions, plating aliquots onto nutrient media, incubation, and counting the resulting individual colonies, which is expressed as n·10m CFU/mL. Meanwhile, in some studies, these results are reported as n·10m CFU/mL per unit of mass (CFU/g) [18], and in others, even as CFU/kg [19], necessitating recalculation for subsequent data comparison. Since liquids can vary in their total soluble solids content, comparing microbiological research results appears more reliable when calculated in CFU/g.

Recently, expressing colony-forming unit counts as n·log10CFU/mL or CFU/g has become widely adopted [20]. While this approach somewhat simplifies the presentation of research data, it nevertheless complicates the comparative analysis of results obtained by other researchers, such as those reported as n·10m.

Variations in the composition of nutrient media significantly hinder the comparative analysis of microbiological findings. For instance, in a study by Marutpong Panya et al., the prebiotic properties of non-digestible carbohydrates were evaluated through a relative comparison of results obtained on a glucose-free medium supplemented with fructooligosaccharides and galactooligosaccharides versus a glucose-containing control medium [21].

Provided that uniform approaches to microbiological testing methods and data calculation are maintained, it becomes possible to determine relative metrics, such as the prebiotic index (PI), which was first proposed by R. Palframan, G.R. Gibson, and R.A. Rastall in 2003 [22]. The essence of this determination involves calculating the ratios of cultivated bacteria of the genera Lactobacillus and Bifidobacterium, as well as bacteroides (Gram-negative, rod-shaped bacteria belonging to the family Bacteroidaceae) and bacteria of the genus Clostridium, grown on the same nutrient medium, to the total number of microorganisms grown on that identical medium [22]:

$$PI=(\text{Bif.})/(\text{Total})-(\text{Bac.})/\text{Total}+(\text{Lac.})/\text{Total}-(\text{Clos.})/\text{Total} \quad (1)$$

where Bif. – Bifidobacterium colony count;
Bac. – Bacteroidaceae colony count;
Lac. – Lactobacillus colony count;
Clos. – Clostridium colony count.

As indicated by the formula, the colony counts of bacteria belonging to the genera Bifidobacterium and Lactobacillus increase the prebiotic index (PI) value, whereas the colony counts of the family Bacteroidaceae and the genus Clostridium decrease it. This is entirely

logical, as prebiotics also serve as an effective nutrient medium for the latter groups [23, 24].

Although the proposed method for evaluating the prebiotic properties of food and feed products is widely applied today [25], from our perspective, the combined determination of the growth of both Bifidobacterium and Lactobacillus fails to yield a reliable prebiotic index value. This limitation arises because these bacteria can exhibit distinct growth patterns upon the incorporation of the investigated components into the nutrient media.

The objective of the study. The objective of this study was to improve the method for evaluating the prebiotic potential of both raw materials and finished food and feed products.

Objects of research. The objects of research were the growth data of bacteria belonging to the genera Bifidobacterium and Lactobacillus cultured on various nutrient media, obtained across different laboratories, including the Microbiology Laboratory of Odesa National University of Technology.

Research materials and methods

Plant-based food and feed raw materials were used as the research materials. The study utilized the following bacterial strains: *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, *Lactobacillus acidophilus*, and *Lactobacillus plantarum*. Corn-lactose medium was used as the nutrient medium for cultivating *Bifidobacterium* species, while MRS medium, currently standard in the vast majority of laboratories, was employed for cultivating *Lactobacillus* species. Traditional microbiological techniques, including the inoculation of the specified bacterial cultures, were applied to determine the prebiotic index. The experimental procedure also involved plating, incubation in a thermostat, and microbial cell counting.

Results of the research and their discussion

To evaluate the prebiotic properties of various types of food raw materials and food products, we propose the separate determination of prebiotic indexes for bacteria of the genera Bifidobacterium and Lactobacillus using the following formulas:

$$PI_{bif} = \frac{Bif_i}{Bif_{contr}}, \quad (2)$$

$$PI_{lac} = \frac{Lac_i}{Lac_{contr}}, \quad (3)$$

where $PI_{bif.}$ – prebiotic index for the genus Bifidobacterium;

$P_{lac.}$ – prebiotic index for the genus Lactobacillus;

Bif_i – Bifidobacterium bifidum CFU count in the nutrient medium with the tested product, $n \cdot 10^m$/g;

Bif_{contr} – CFU count of Bifidobacterium bifidum on standard MRS nutrient medium MRS, $n \cdot 10^m$/g;

Lac_i – CFU count of Lactobacillus acidophilus upon inoculation into the medium supplemented with the tested product, $n \cdot 10^m$/г;

$Lac_{contr.}$ – CFU count of Lactobacillus acidophilus on corn-lactose nutrient medium.

This approach enables the acquisition of comparable results regarding the prebiotic potential of a given food product, as the proposed prebiotic index evaluates the impact of the investigated product or its ingredient relative to a control – namely, the nutrient medium on which each bacterial species of the genera *Bifidobacterium* and *Lactobacillus* exhibits optimal growth. Consequently, it is highly advisable to always specify the exact type of nutrient media used when reporting research results.

Table 1 presents the research results for the growth of bacteria belonging to the genera *Bifidobacterium* and *Lactobacillus*, expressed as $n \cdot 10^7$ CFU/g [26]. Based on these data, we calculated the prebiotic index, which is also provided in Table 1.

Table 1 – Determination of the prebiotic index of vegetable processing by-products

№	Sample	*Lactobacillus plantarum* 10^7 CFU/g	Prebiotic index $PI_{lac.}$	*Bifidobacterium adolescentis* 10^7 CFU/g	Prebiotic index $PI_{bif.}$
1.	Control sample	1,60	-	2,30	-
2.	Centrifugal carrot pomace	1,12	0,7	1,10	0,48
3.	Screw-pressed carrot pomace	3,20	2,0	1,10	0,48
4.	Centrifugal pumpkin pomace	1,36	0,85	2,70	1,17
5.	Screw-pressed pumpkin pomace	1,04	0,65	2,50	1,09
6.	Centrifugal beetroot pomace	1,44	0,9	1,20	0,52
7.	Screw-pressed beetroot pomace	2,08	1,3	2,80	1,22

The separate determination of prebiotic indexes allows for a clear differentiation of the raw material's functional effects. This is clearly supported by the data in Table 1. For instance, carrot pomace obtained via screw pressing demonstrates a pronounced prebiotic effect toward *Lactobacillus plantarum* ($BI_{lac.}$= 2,0), but proves ineffective for *Bifidobacterium adolescentis* ($BI_{bif.}$= 0,48). An integral index would have concealed this specificity, thereby complicating the targeted design of products tailored to specific dysbiotic states and the physiological needs of both humans and animals.

The obtained results also demonstrate a clear dependence of the prebiotic index on the method of mechanical processing applied to the vegetable raw materials. Specifically, for carrot and beet pomace obtained via screw pressing, a significant increase in microbial growth is observed compared to their centrifugal counterparts. This phenomenon has a profound biochemical rationale. Screw pressing, as opposed to centrifugation, is accompanied by more intense shear deformations and mechanochemical destruction of plant cell walls. This facilitates the partial depolymerization of poorly soluble oligosaccharides into soluble ones with a lower degree of polymerization, making them more accessible for hydrolysis by bacterial enzymes (endo- and exo-glycosidases).

Table 2 presents the research results for the growth of bacteria belonging to the genera *Bifidobacterium* and *Lactobacillus*, expressed exclusively as $n \cdot 10^6$ КУО/г. The difference in the order of magnitude of the numbers presented in Table 1 and Table 2 did not hinder the comparative analysis.

Thus, Table 2 outlines the data regarding the determination of the prebiotic index for common vegetables.

The results of determining the prebiotic index of common vegetables demonstrate that the studied samples exhibit high selectivity regarding the growth stimulation of individual microbial genera. None of the tested products simultaneously displayed maximum prebiotic index values for both lacto- and bifidobacteria, which experimentally confirms the validity of applying the developed approach for the separate calculation of indexes (($BI_{lac.}$ та $BI_{bif.}$).

The study also revealed an antagonistic or inhibitory effect of white onion toward *Bifidobacterium bifidum* ($BI_{bif.}$= 0). Given that this vegetable is renowned for its high content of prebiotic carbohydrates (inulin and fructooligosaccharides), the resulting zero growth may indicate a pronounced sensitivity of this particular bifidobacterial strain to the phytoncide fractions (essential oils, allicin) or flavonoids of the white onion, which inhibit their development *in vitro*. Concurrently, red salad onion proved to be safe for bifidobacteria ($BI_{bif.}$ = 0,89), indicating a substantial dependence of the raw material's bioactivity on its varietal characteristics and component composition.

Among the investigated raw materials, eggplant demonstrated the best balance in stimulating both groups of microorganisms ($BI_{lac.}$ = 0,92; $BI_{bif.}$ = 0,86) and carrot ($BI_{lac.}$ = 0,72; $BI_{bif.}$ = 0,68). Although their prebiotic indexes are slightly lower than those of the control, the stable growth rates of both test cultures indicate the uniform availability of the polysaccharide complex of these vegetables (specifically pectin and fiber) for fermentation by both lacto- and bifidobacteria.

The minimum prebiotic index values were recorded for red bell pepper ($BI_{bif.}$ = 0,05) and Zucchini ($BI_{bif.}$= 0,21). This indicates a deficit of specific oligosaccharide fractions within their matrix required to sustain the bifidobacteria population, making this raw material less promising for use as a standalone prebiotic base in functional food technologies without additional modification.

Thus, the results of the microbiological studies presented in Table 2 provide a scientific rationale for the formulation modeling of a wide range of multicomponent functional products. Specifically, to develop products with a targeted lactogenic effect, it is advisable to use white cabbage as a base recipe component, whereas combining beetroot with salad varieties of red onion is promising for stimulating bifidoflora $BI_{bif.}$= 0,61, and under conditions of screw pressing of beetroot pomace, $BI_{bif.}$= 1,22).

Table 2 – Determination of the prebiotic index of common vegetables

№	Product	*Lactobacillus acidophilus* 10^6 CFU/g	Prebiotic index $PI_{lac.}$	*Bifidobacterium bifidum* 10^6 CFU/g	Prebiotic index $PI_{bif.}$
1.	Control sample	25	-	28	-
2.	White onion	31	1,24	0	0
3.	Red onion	15	0,60	25	0,89
4.	Early potato, «Riviera» variety	26	1,04	16	0,57
5.	Beetroot	27	1,08	17	0,61
6.	Red bell pepper	18	0,72	1,5	0,05
7.	Zucchini	15	0,60	6	0,21
8.	Eggplant	23	0,92	24	0,86
9.	Carrot	18	0,72	19	0,68
10.	White cabbage	37	1,48	10	0,36

Conclusion

As a result of the conducted research, we propose determining the prebiotic indexes of food and feed raw materials, as well as finished food and feed products, separately for the bacterial genera *Bifidobacterium* and *Lactobacillus*. The scientific approach of calculating separate prebiotic indexes for *Bifidobacterium* and *Lactobacillus* species represents a significant advancement in nutritional science, contributing to the expansion of the functional food and feed product range. The classical prebiotic index is an integral metric that overlooks the specific substrate selectivity of microorganisms. Given that lactobacilli and bifidobacteria possess fundamentally distinct enzymatic pathways for carbohydrate biotransformation, their responses to the identical oligosaccharide vary substantially. It has been established that the proposed approach facilitates seamless comparative analysis of the prebiotic potential across various types of raw materials and ready-to-consume food and feed products..

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ПРОБЛЕМИ ОЦІНКИ ПОТЕНЦІЙНОЇ ПРЕБІОТИЧНОЇ АКТИВНОСТІ ХАРЧОВИХ ТА КОРМОВИХ ПРОДУКТІВ

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Анотація. Розвиток наукових досліджень у напрямі створення нових функціональних продуктів харчування та кормових добавок зумовив значне розширення їхнього асортименту. Вибір ефективних інгредієнтів для таких продуктів, а також визначення впливу подальших технологій переробки потребують оцінювання рівня їхньої функціональності, що пов'язано з істотними витратами часу та матеріальних ресурсів. Тому застосування методів оцінки потенційних пребіотичних властивостей харчової та кормової сировини, а також готових до споживання продуктів становить значний інтерес як для науково-дослідних, так і для виробничих лабораторій. У 2003 році Р. Палфраман, Г. Р. Гібсон та Р. А. Расталл запропонували метод визначення пребіотичного індексу (PI). Цей метод передбачає розрахунок співвідношення культивованих бактерій родів *Lactobacillus* і *Bifidobacterium*, а також бактероїдів родини *Bacteroidaceae* та бактерій роду *Clostridium*, вирощених на одному повноцінному середовищі, до загальної кількості мікроорганізмів, що виросли на цьому ж середовищі. У цій роботі зроблено спробу вдосконалити згаданий метод визначення пребіотичного індексу шляхом окремого розрахунку співвідношення культивованих бактерій родів *Lactobacillus* і *Bifidobacterium* до бактерій тих самих родів, вирощених на ідентичних контрольних поживних середовищах. Окреме оцінювання пребіотичних індексів дає змогу чіткіше диференціювати функціональні ефекти сировини порівняно з інтегральним пребіотичним індексом, який усереднює пребіотичні характеристики, що ускладнює цільове проектування продуктів для конкретних дисбіотичних станів та потреб організму людини і тварин. Визначення пребіотичного індексу за ростом бактерій *Bifidobacterium* і *Lactobacillus* при додаванні зразків поширених овочів до контрольних поживних середовищ підтвердило високу селективність щодо стимуляції росту окремих родів мікроорганізмів. Жоден із протестованих продуктів не виявив одночасно максимальних значень пребіотичного індексу як для лакто-, так і для біфідобактерій, що експериментально підтверджує обґрунтованість розробленого підходу щодо роздільного розрахунку індексів (BI_{lac} та BI_{bif}).

Ключові слова: функціональні продукти харчування та корми, пребіотичні властивості, пребіотичний індекс, біфідобактерії та лактобацили, овочі, вичавки.

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