UNIWERSYTET EKONOMICZNY W POZNANIU

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Design of an innovative fermented product using kale juice as an example

Summary of doctoral dissertation

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Poznań 2022

1. Justification of the addressed research problem

The development of new food products, in particular health-enhancing foods, is increasingly the subject of scientific interest, and the practical activities undertaken in this area are on an unprecedented scale (Horvat et al., 2019). The process of product design and development, due to its complexity, is a costly and time-consuming endeavor as well as highly risky for failure. Nevertheless, companies can minimize the risk of failure by implementing activities related to the efficient and effective management of the new food product development process (Boroń, 2010; Pinna i in., 2018).

The food industry, ranging from agriculture to food services, is one of the largest industries in the world, playing a key role in many countries (Meiselman, 2007). In the European Union, it is considered one of the most significant in the current economy and therefore attracts the attention of various actors, among others, national authorities and national and international organizations, due to the assurance of safety and high quality products (FoodDrinkEurope, 2020; Pinna et al., 2018). Data show that the industry is important for the European economy, accounting for as much as 15.2% of the manufacturing industry (FoodDrinkEurope, 2020). The food industry is a mature industry that, as a result of numerous transformations, has been dominated by a small group of the few largest players (Bigliardi and Galati, 2013). This is indirectly influenced by the numerous changes in manufacturing technologies, market patterns and health-oriented consumer demands that have been observed over recent years. Therefore, companies operating in the food industry are somehow forced to focus their resources on innovation and new product development in order to maintain or gain a competitive advantage (Bigliardi and Galati, 2013; Pinna et al., 2018).

The need for food companies to undertake new product development activities is determined by the situation prevailing in the industry as a whole. Applying the classical concept of competition existing in the industry (Porter, 1980; Porter, 2008) proves that in such a situation enterprises are often forced to undertake strategies based on non-price competition. Such competition can take different forms and requires companies to take appropriate actions in the area of management (Widuri and Sutanto, 2019). The differentiation strategy, the introduction of which in the enterprise may be a response to the need of non-price competition, consists in differentiation of the product offer (Jurek-Stępień and Wysocki, 2007). In turn, the basis for differentiation of the offer may be the development of new food products.

Given that a new product's success in the marketplace requires that it be accepted by consumers, it is important to place consumers at the heart of the new product development process (Anwar, 2016). A central pillar of this approach is the assumption that consumer needs should be the starting point for new product development processes, and that the purpose of R&D should be to meet consumer needs rather than to develop products as such (Costa and Jongen, 2006). In this case, the success of the new product development process is measured by the degree to which the final

product meets consumer needs. This approach is particularly relevant to consumer goods manufacturers, as it focuses on using knowledge about end users in the development process (O'Sullivan, 2017).

Adapting products to consumer preferences requires companies to have and use knowledge about prevailing consumer trends in the market. Research conducted in this area indicates that one of the dominant dietary trends today is the trend related to healthy eating (Horvat i in., 2019). The scientific literature indicates that foods with added health value offer significant growth opportunities for food companies (Kleef i in., 2005). Therefore, it can be assumed that products with functional properties have a good chance of being accepted by consumers and thus of achieving market success. A functional food is one that beneficially affects one or more target functions in the body in addition to having an adequate nutritional effect, and is not a pill, capsule or any form of dietary supplement (FUFOSE, 1999). Functional products require individual ingredients to be directly linked to specific, well-defined health effects, and these effects to a specific product (Lahteenmaki, 2003). This requires scientific proof of the health effects of a product through laboratory studies (Tsimiklis i in., 2015).

Importantly, consumers do not perceive functional foods as a homogenous product category (Urala i Liisa, 2004). Consumer acceptance of such foods depends on the characteristics of the base product that serves as a vehicle for the functional ingredient or a persuasive message communicating health benefits. Research shows that consumers are particularly interested in functional foods developed from products they perceive as healthy, such as fruit and vegetable products (Balasubramanian i Cole, 2002).

There is currently a wide range of functional products available on the market. Companies using a differentiation strategy are therefore actively looking for market niches that can be developed. Based on market analysis, it can be concluded that a certain niche in the food market is fermented products. This applies in particular to fermented beverages, which, despite their availability to consumers, have a marginal market share (European Commission, 2016). At present the market seems to be dominated by dairy fermented products, although non-dairy fermented products based on fruit and vegetables are attracting increasing attention from various groups - scientists, food producers and consumers. This trend is particularly related to consumers' growing awareness of the health benefits of consuming pickled products. Additionally, many people are changing their dietary habits, including switching to a vegan or vegetarian diet, avoiding the consumption of cow's milk or lactose, and limiting the consumption of dairy products high in cholesterol (Granato et al., 2010, 2020; Panghal et al., 2018)

The identification of this market niche is at the same time consistent with existing observations on functional products, since, according to the current state of knowledge, additional health-promoting properties beyond the nutritional effect can be observed due to the changes occurring during lactic fermentation. As indicated by scientific studies, the consumption of fermented fruit and vegetable juices

(usually containing probiotic microorganisms), can contribute to the health of consumers by, among other things: modulating glycaemia(Gao et al., 2019), regulating body fat (Verón et al., 2019) and anti-inflammatory activity (Filannino et al., 2013). The selection of starter cultures is also an important aspect, including their potential probiotic properties, determining the functional characteristics of the fermented product. The synergistic action of probiotic microorganisms and plant material may contribute to the maintenance of a healthy and balanced intestinal microbiota and influence immune defense mechanisms (Marco et al., 2021).

In an attempt to select plant material on the basis of which a new product could be developed, a market analysis was carried out including the offer of fermented juices and the characteristics of the research material. The present study proposes green kale as a base for the creation of a fermented juice using indigenous lactic fermentation bacteria with probiotic potential. In recent years, this vegetable has gained great popularity as a so-called '*superfood*' due to its high content of bioactive compounds. The properties of kale have been well documented in the literature, in particular its potential properties to prevent diabetes, cardiovascular disease or cancer (Becerra-Moreno et al., 2014; Biegańska-Marecik et al., 2017; Olsen et al., 2010; Šamec et al., 2018). The health-promoting effects of kale are closely related to its high content of vitamins: C, K and A, as well as niacin (B_3), pyridoxine (B_6), riboflavin (B_2) and folic acid (B_9). This vegetable is also characterized by a significant content of glucosinolates (e.g. glucoiberin, sinigrin), phenolic compounds (e.g. caffeic acid, quercetin, kempherol) and carotenoids (e.g.β -carotene, lutein). In addition, kale is a good source of macro- and microelements such as potassium, calcium, magnesium, iron, copper and manganese (Šamec i in., 2018; Szutowska i in., 2020; Thavarajah i in., 2016).

Therefore, it has been concluded that **the development of new products** is an important activity for companies operating in the food market, and a potentially important direction of development is **functional food** (which fits into consumer preferences related to healthy eating) **created on the basis of vegetables and fruit** (material which is perceived as healthy) and **subjected to the process of lactic fermentation** (fermented food has a relatively small market share). Fermented green kale juice can be used as an example.

However, it is important to highlight that there is limited research dedicated to new product development in the food industry with a focus on fermented products. The research gaps identified in the literature relate firstly to the new product development process itself, defined as the process of transforming a new market opportunity into a commercial product through a specific sequence of actions (Azanedo et al., 2020; Krishnan and Ulrich, 2001; Rudder et al., 2001). Numerous models for the development of new, innovative products can be identified in the scientific literature (Bernstein and Singh, 2006; Cooper, 2008; Hallstedt et al., 2013; Louw et al., 2018; Rogers, 2003). However, there is no unanimity concerning the model of new product development dedicated to food enterprises and taking into account the characteristics of the industry. This shortage of knowledge represents

an important and current constraint on the road to improving the quality of the management of development processes in the food industry. Moreover, managing new product development requires knowledge of consumer behaviour. The literature in this area shows a gap concerning the relationship between demographic and social factors such as gender age, material status and place of residence and the frequency of consumption (Antonio and Gonzalez, 2009; Büyükkaragöz et al., 2014; Karelakis et al., 2020; Kraus et al., 2017; Rivas-Rojas and Cuffia, 2020). The literature in this field shows a gap concerning the relationship between demographic and social factors such as gender, age, material status and place of residence and the frequency of consumption, as well as a gap relating to the behavior of Polish consumers of non-dairy fermented products. This is a limitation at the initial stages of the new product development process, where the whole process is directed in the design of a new product. In addition, the development of new functional products requires advanced laboratory research. In this regard, significant shortcomings have been identified in research on the comprehensive characterization of fermented green kale juice. Few attempts have been made in the scientific literature to investigate the potential of green kale, e.g. in freeze-dried form as an additive to apple juice to increase the health-promoting properties of the product (Biegańska-Marecik et al., 2017) in the form of fermented juice used in the production of feta-type cheese (Michalak, Skrzypczak, et al., 2020) or as a source of gentisinic and salicylic acids with anticancer properties (Michalak, Szwajgier, et al., 2020). However, there are still many areas that can complement the previous considerations, including microbiological analyses (focused on the quality of the research material, antimicrobial properties and the possibility of isolation and selection of lactic fermentation bacteria with potential probiotic properties), and instrumental analyses based on the identification of biologically active compounds (e.g. content of vitamins, phenolic compounds, glucosinolates).

2. Objectives and research hyphotheses

The process of new food product development, as already mentioned, is complex and multi-stage. It takes into account market expectations, consumer preferences, current trends and, on the other hand, makes use of the company's capabilities and numerous laboratory tests. When creating a new fermented product characterized by functional properties, many aspects must be considered, including the possibility of shaping the quality of the product (e.g. the content of various bioactive compounds) by various microorganisms with probiotic potential and consumer studies outlining the nature of the product.

With reference to the considerations presented in the first two chapters, the main objective of this dissertation was defined. The main objective of this thesis was: **to design and develop a prototype of a new food product by fermenting kale juice using selected strains of lactic fermentation bacteria.**

In addition to the main objective, the following specific objectives (CS) have been formulated and are presented in Table 1.

Table 1. Specific objectives of the dissertation

L.p.	Specific objective (CS)	Reference in the dissertation
CS 1	Developing a model approach to new food product development	**Chapter 2, subchapter 2.3.**
CS 2	Determining Polish consumers' behavior concerning non-milk fermented products	**Chapter 4, subsection 4.1.1.**
CS 3	Determination of selected health-promoting properties of spontaneously fermented green kale juice	**Chapter 4, subsection 4.2.1**
CS 4	Isolation of lactic fermentation bacteria from the microbiota of green kale during successive stages of spontaneous fermentation	**Chapter 4, subsection 4.2.2**
CS 5	Characterization of probiotic potential and molecular identification of selected isolates of lactic fermentation bacteria.	**Chapter 4, subsection 4.2.2**
CS 6	Development of prototypes of fermented green kale juices	**Chapter 4, subsection 4.3.1**
CS 7	To determine the effect of fermentation using selected bacterial strains on selected physicochemical parameters, nutritional and health-promoting properties of pickled juice made from green kale.	**Chapter 4, subsection 4.3.2**

Source: own elaboration

Based on the analysis of the literature data, the research hypotheses were formulated and are presented in Table 2.

Table 2. Main and partial hypotheses of the dissertation

L.p.	Main hypotheses (H)	Reference in the dissertation
H1	Demographic factors determine consumer choices in the consumption of pickled vegetable products.	**Chapter 4, subsection 4.1.1.**
H2	Spontaneously fermented kale juice has a higher content of bioactive compounds than fresh juice.	**Chapter 4, subsection 4.2.1**
H3	Selected strains of lactic fermentation bacteria isolated during spontaneous fermentation of green kale have potential probiotic properties.	**Chapter 4, subsection 4.2.2**
H4	Kale microbiota is a source of lactic fermentation bacteria useful as starter cultures for modelling selected health-promoting properties of fermented green kale juice.	**Chapter 4, subsection 4.3.2**
H5	Controlled fermentation of kale juice allows for a higher content of selected bioactive components compared to fresh juice and juice subjected to spontaneous fermentation.	**Chapter 4, subsection 4.3.2**

Source: own elaboration

3. Structure of the dissertation

The first chapter presents trends in the food market, including fermented food in particular. The individual subchapters present considerations on the intensification of competitive struggle in the food industry, as well as the basis for competition on the basis of differentiation strategies.

The importance of effective management of new product development was referred to, and the important role of consumers in the process was initially signaled. On the basis of market analyses, the currently dominating nutritional trend on the market related to healthy eating was indicated and characterized. The next subchapter defines functional food and discusses the characteristics of fermented food. First of all, their health-promoting properties, resulting mainly from the presence of biologically active compounds and beneficial microorganisms, were pointed out. Finally, the first chapter identifies and discusses the niche on the food market which is represented by fermented products, especially fermented beverages. Innovations in the food market and innovations in the fermented food market are characterized and a rationale for the need to actively manage the development of new products in the food market is presented.

The second chapter focuses on the development of new products in the food industry. In the first subchapter, the requirements for fermented products are presented, including first of all the European and Polish regulations on the food market. In the next part, the scientific basis of the concept of new product development is discussed, indicating that the main pillar of the concept is that consumer needs should be the starting point for new product development processes. In the third subchapter, the author's model of new food product development was developed. The methodology of conducting systematic literature studies is presented, followed by the author's model of new product development. The model includes seven distinctive stages: idea generation, idea selection, research, development, testing, market launch, monitoring. In the last subsection, the considerations focused on the management of the new product development process from the project perspective. Based on the methodology proposed by the Project Management Institute (PMI), examples of management on the basis of the development of fermented green kale juice were presented.

Chapter three presents the research objectives and hypotheses and presents the research methodology. The empirical research included both consumer and laboratory research. With reference to consumer research, the methodology of conducting questionnaire studies is presented. On the other hand, with reference to laboratory research, the research material, reagents and indicator microorganisms were discussed. Next, the methods used for microbiological and instrumental tests are presented in detail. Finally, methods used in statistical data analysis are presented.

The fourth chapter presents the results of the own research and the discussion. This chapter has a precisely planned structure. This structure has been designed to be consistent with the elaborated model of new food product development. Subsequent subchapters therefore correspond to selected subsequent stages distinguished in the model. The chapter is divided into three subchapters: idea generation and selection, research and development. The first subchapter presents the results of conceptual work and empirical research aimed at developing the concept of a new food product and determining the behavior of Polish consumers regarding non-lactic fermented products. The second subchapter presents the results of health promoting properties and quality characteristics

of spontaneously fermented kale juice, as well as potential probiotic properties of lactic fermentation bacteria. In the last subsection, results on the development of fermented juice prototypes are presented and studies covering nutrients and health-promoting properties of juices subjected to controlled fermentation are presented.

The paper concludes with a summary that answers the research question and discusses the results of hypothesis testing. Limitations of the results obtained, possible directions for further research and managerial implications for food companies are also presented.

4. Research methodology

A variety of research methods listed and characterized in Table 3 were used to achieve the planned objectives and verify the research hypotheses. A variety of research methods were used within this dissertation, including systematic literature review methods, microbiological analysis, instrumental analysis, survey research, and statistical analysis

Table 3. Research methods

L.p.	Method
	Systematic literature review
1.	A systematic literature review was conducted using: (a) SALSA methods (Booth, Papaioannou, i Sutton, 2012), (b) *backward snowballing* procedures) (Jalali i Wohlin, 2012), (c) content analysis.
	Microbiological methods
2.	The classical Koch plate method using different multiplication and selective media was used to monitor the microbiological quality of the test material, the isolation and the abundance and viability of lactic fermentation bacteria.
3.	Macro- and microscopic analyses were used for preliminary characterization of the lactic fermentation bacteria isolated from the study material.
4.	Isolation of genomic DNA from bacteria was performed using a ready-made isolation kit according to the protocol from the manufacturer (Genomix Mini AX Bacteria+ Spin, A&A Biotechnology, Poland).
5.	Amplification of the 16s rRNA gene (polymerase chain reaction and electrophoretic analysis) was performed to duplicate the genetic material necessary for bacterial identification (Leite et al., 2015).
6.	Sequencing of the 16s rRNA gene allowed genetic identification of the isolates (Genomed S.A.).
7.	Identification of bacterial isolates was performed using a MALDI-TOF mass spectrometer (Jagiellonian Centre for Innovation) (Banach et al., 2016).

8.	The construction of a phylogenetic tree using the *neighbour-joining method* enabled the determination of the relatedness of the selected strains and the verification of the correctness of the identifications carried out (Kumar et al., 2018; Saitou and Imanishi, 1989).
9.	The well diffusion method was used to evaluate the antimicrobial activity of isolates of lactic fermentation bacteria.
10.	The disc method was used to assess the susceptibility of bacterial isolates to selected antibiotics (CLSI, 2019; Rubio et al., 2014).
11.	The microplate method using sodium chloride, hydrochloric acid and bile salts made it possible to determine the survival of selected isolates of lactic fermentation bacteria under simulated gastrointestinal conditions and in the presence of NaCl concentration.
12.	The double dilution method on microplates was used to evaluate the antimicrobial activity of fermented juices (Niemczak et al., 2019).
Instrumental and physicochemical methods	
13.	The methods of atomic emission spectroscopy with microwave plasma and atomic absorption spectroscopy with graphite cuvette enabled the measurement of macro- and microelements and heavy metals (Abid et al., 2014; Anastácio et al., 2018).
14.	The TEAC (*Trolox Equivalent Antioxidant Capacity*) method was used to evaluate the antioxidant activity of the juices studied (Re et al., 1999).
15.	The Folin-Ciocalteu method made it possible to determine the total content of phenolic compounds in the juices studied (Singleton and Rossi, 1965; Włodarska et al., 2017).
16.	The pH measurements allowed the monitoring of changes in the acidity of the juices tested.
17.	The refractometric method made it possible to measure the total content of soluble solids.
18.	The titration method made it possible to determine the acidity of the juices studied (Polish Committee for Standardization, 2013).
19.	High performance liquid chromatography (HPLC) was used to determine the content:
	• vitamin C (Gliszczyńska-Świgło and Tyrakowska, 2003; Kurilich et al., 1999);
	• selected B group vitamins (Gliszczyńska-Świgło and Rybicka, 2015);
	• fructose and glucose (Rybicka and Gliszczyńska-Świgło, 2021; Shanmugavelan et al., 2013);
	• selected phenolic compounds (Tsao and Yang, 2003; Vallejo et al., 2002);
	• selected carotenoids (de Sá and Rodriguez-Amaya, 2004);
	• selected glucosinolates (EN ISO 9167:2019, 2019).
20.	Measurement of water, ash, protein, fat, carbohydrate content used to determine the nutritional value of juices (AACC, 2009; ISO, 2007, 2013).
21.	The UV-VIS spectrophotometric method made it possible to determine changes in the color of the juices.

Survey method	
22.	A survey, using a questionnaire specially designed for this purpose, explored consumer choices for fermented vegetable products.
Statistical analyses	
23	• Determination of means and standard deviations (Excel MS Office). • A one-way analysis of variance (ANOVA) allowed comparison of mean scores between samples/factors (SPSS Statistics). • Ordinal regression (SPSS Statistics).

Source: own elaboration

5. Research results and conclusions

The aim of this dissertation was to **design and develop a prototype new food product by fermenting kale juice using selected strains of lactic fermentation bacteria**. The whole research process was based on the concept of new product development considering the specifications for food product design. The presented dissertation, focusing on the comprehensive characterization of fermented kale juice on the basis of the new food product development concept, required the application of multidirectional research methods, i.e. a systematic literature review including the analysis of new food product development models, survey research on consumer behavior in the non-lactic fermented food market, and microbiological, physicochemical and instrumental methods focusing on the determination of health-promoting properties and quality distinguishing features of fermented green kale juice.

A series of analyses covering the concept of development of a new fermented product has significantly enriched the existing state of knowledge both in the theoretical and empirical layers. In the theoretical layer, the model of new food product development developed, by considering the specificity of the industry, complements the current state of knowledge in the field of project-based management of the new product development process. On the other hand, in the empirical layer, numerous laboratory studies indicate the possibility of an innovative use of green kale juice in the form of a fermented product with functional properties including probiotic properties and high content of bioactive compounds.

In order to develop a model for new food product development, a systematic literature review was conducted covering different approaches to new product development in the food industry. The model includes seven distinctive stages: idea generation, idea selection, research, development, testing, launch and monitoring. The model adopts a process perspective based on sequential logic, where successive stages form a coherent sequence. The model's composition assumes that in order to develop a new food product, a sequence of logically separated activities must be completed. The model assumes that all stages are interrelated and that the process can proceed both from earlier to later stages and can be taken back from later stages to earlier stages (e.g. unsuccessful testing can

take the product back to the development stage). Within the paper, all stages of the process are characterized in detail:

1. **Idea generation** - using all available internal and external sources of ideas for new products.
2. **Idea selection** - selection of ideas based on established evaluation criteria including analysis of the environment and company strategy.
3. **Research** - conducting both basic research (focused on acquiring new knowledge) and applied research (focused on specific and predetermined target applications).
4. **Development** - using existing knowledge gained during the research phase to produce a prototype product.
5. **Testing** - conducting mandatory quality tests and determining the position of the new product on the market.
6. **Market launch** - production of new products and possible adaptation of the current production process, as well as the introduction of products on the market.
7. **Monitoring** - gaining experience, recording qualitative and quantitative data and performing analysis to expand the company's knowledge base.

In relation to the first two stages of the process - **idea generation and selection**, it was found that one of the currently dominant trends in the food industry is the trend related to healthy eating, and products with health-promoting properties have a particularly high chance of being accepted by consumers and thus of market success. It was explained on the basis of the theory of planned behavior (Ajzen, 1991). It is explained that due to the social impact of initiatives promoting healthy eating behavior, there is a change in attitudes, subjective norms and a feeling of control over consumers' ability to eat a healthy diet, and thus consumers' health-seeking behavior will remain at a given level or increase in the future.

The development of functional foods, understood as foods that beneficially affect one or more target functions in the body in addition to their respective nutritional effects, offers significant growth opportunities for food companies. When analyzing the possibilities of targeting the development of new functional products, it was found that consumers do not perceive functional foods as a homogeneous product category and the possible acceptance of a new product depends on the characteristics of the base material. Consumers are particularly interested in functional foods developed from material they perceive as healthy (e.g. vegetables). However, the development and marketing of functional foods is expensive, risky and involves a lot of laboratory testing, and managing their development is therefore particularly demanding.

Taking the differentiation strategy as a basis, it was determined that in order to differentiate themselves effectively, companies should develop new functional products based on products that have had little presence in the market in the past. Fermented products, including fermented beverages in particular, represent a niche. It has been established that the market is dominated by dairy fermented

products, and thus the development of non-dairy fermented products based on fruit and vegetables is a particularly important potential direction for new product development. Fermented foods can be categorized as functional products due to the changes that occur during lactic fermentation (bioconversion of biologically active compounds) and due to the presence of microorganisms with probiotic potential. Consequently, regular consumption of fermented products contributes to improving the health of consumers (Marco et al., 2021; Septembre-Malaterre et al., 2018).

Conducting literature studies and analysis of fermented products available on the market made it possible to select a valuable plant material - green kale, on the basis of which a product meeting the criteria defined above could be developed. It is worth noting that currently on the market there is a lack of products based on fermented kale juice, despite the fact that the offer includes various pickled vegetable-based juices. The selection of material was also based on the characteristics of kale. This vegetable is characterized by a high content of bioactive compounds (vitamins, phenolic compounds, carotenoids, glucosinolates) and minerals, which have positive effects on human health. Due to its content of these compounds, kale has numerous health-promoting properties that include the prevention of chronic degenerative diseases, cardiovascular diseases and the formation of cancer (Šamec et al., 2018; Thavarajah et al., 2016).

In addition to the above considerations, a further part of the research work within idea generation and selection involved conducting consumer research. This was important because it is the consumers' opinion that partly influences the commercial success of the product. The study found that pickled vegetable products are associated primarily with specific products (cabbage and cucumbers), health properties and a characteristic taste. According to the respondents, their main advantages are health-promoting properties, probiotic properties and longer shelf life. The study found that pickled vegetable juices enjoy a moderate frequency of purchase among respondents, lower than other pickled vegetable products. As the single most important criterion for choosing pickled vegetable juices, respondents indicated taste (48%), followed by health-promoting properties (46%), product composition (45%), probiotic properties (43%) and nutritional value (39%). In addition, respondents felt that the range of fermented vegetable juices could be further expanded. Regarding pickled vegetable juices, respondents pointed out disadvantages such as low availability, inappropriate taste or smell and high price. When indicating their advantages, they most often mentioned their health-promoting nature, unique taste and long shelf life.

By verifying the first hypothesis, it was found that demographic factors do not determine consumer choices in the consumption of fermented vegetable preserves. However, narrowing down the study to fermented vegetable juices allowed us to show that these factors are important for this product category. When examining pickled vegetable juices specifically, it was found that women were 0.856 times more likely to consume pickled vegetable juices than men. On the other hand, the chance that young people (aged 21-30) are more likely to consume pickled vegetable juices is more than double

(- 2.305) compared to people aged 60 and above. On the other hand, people living in cities with a population between 20 000 and 99 000 are 0.849 times more likely to consume pickled vegetable juices than those living in major urban centers (over 500 000).

> ***The first*** *hypothesis stated that demographic factors determine consumer choices in the consumption of fermented vegetable products-was* ***verified positively*** *for fermented vegetable juices and* ***negatively*** *for fermented vegetable preserves.*

In the further part of the work (research and development), the focus was on the technological aspects of developing a new food product - fermented kale juice. Laboratory research included a comprehensive characterization of the fermented juice, which required the application of multidirectional research methods, i.e. microbiological and instrumental methods. Based on the current scientific literature on potential applications of kale, it was concluded that there is a research gap regarding the comprehensive characterization of fermented green kale-based juice. The innovative use of kale in the form of a fermented product significantly enriches the current state of knowledge, as well as provides a novel proposal for the use of kale as a juice with potential health-promoting properties.

In the **basic research** stage, preliminary laboratory analyses were conducted to gain new knowledge on the possibility of spontaneous fermentation of kale juice and to determine its selected quality characteristics. At the same time, it should be emphasized that kale is not traditionally subjected to the fermentation process, thus it is a kind of scientific novelty. The microbiological quality of the raw material and the fermented juice was satisfactory - the presence of selected pathogenic microorganisms was not detected. As the study showed, after 48 hours of the process, the number of lactic fermentation bacteria increased significantly compared to fresh kale. This was crucial for the success of further research stages in the development of the new food product. The higher the number of lactic fermentation bacteria, the higher the probability of species diversity of the isolates and thus the greater the possibility of selecting valuable bacterial strains. Spontaneous fermentation of kale juice contributed to the bioconversion of biologically active compounds, which was manifested by an increase in antioxidant properties and an increase in the total content of phenolic compounds. On the other hand, a decrease in vitamin C content was observed. On the other hand, the level of mineral components remained unchanged. It was further shown that spontaneous fermentation of the juice contributes to potential health-promoting properties by improving antimicrobial properties. The presence of the fermenting microbiota as well as its metabolites is also not negligible as it can have beneficial effects on human health.

*Therefore, **hypothesis two**: Spontaneously fermented kale juice is characterised by a higher content of bioactive compounds than fresh juice was **verified positively** in view of higher antioxidant properties and higher content of phenolic compounds. On the other hand, **a negative verification** was related to a decrease in vitamin C content.*

This was followed by **applied research**, which consisted of target- and application-oriented analyses. The research involved determining the potential probiotic properties of lactic fermentation bacterial isolates. In a first step, 80 strains of lactic acid bacteria were isolated during different process steps. Then, after preliminary characterization, the isolates were subjected to screening tests - their antimicrobial properties, sensitivity to selected antibiotics were determined and they were identified to the species level using the proteomic method.

Based on global guidelines and literature, a methodical selection of the most valuable bacterial strains was made, both in terms of their properties and species diversity. As a result, the conducted research made it possible to select 12 valuable and species-diverse strains of lactic acid bacteria out of 80 isolates. 10 strains of lactic fermentation bacteria were genetically identified to the species level. These microorganisms belonged to the species *L. mesenteroides*, *L. sakei*, *L. plantarum*, L. *coryniformis*. The selected 10 strains can be classified as potential probiotic microorganisms due to: adequate lack of resistance to selected antibiotics, high antimicrobial properties, adequate survival under simulated gastrointestinal conditions, and genetic identification to the strain level.

***The third hypothesis**, that selected strains of lactic fermentation bacteria isolated during spontaneous fermentation of green kale have potential probiotic properties, was **verified positively**.*

At the **development** stage, initial analyses were carried out on 10 pre-selected microorganisms based on bacterial abundance in the juice matrix and efficiency in acidifying the product. This stage enabled the selection of three bacterial isolates, which served as monocultures (*L. plantarum* JS052 and *L. sakei* JS032) as well as mixed cultures (MIX A*: L. mesenteroides* JS027, L. *plantarum* JS052, *L. sakei JS032 and* MIX B: L. *plantarum* JS052 and *L. sakei JS032*) for the actual study - the creation of 4 prototypes of fermented green kale juice. Due to ineffective lowering of juice pH, isolates belonging to *L. coryniformis* species were not included in the creation of product prototypes.

During the lactic acid fermentation process, strain-dependent properties were observed in the bioconversion of biologically active compounds and nutritional properties. Changes in nutritional value were relatively similar for all variants used. The most significant difference was the ability to metabolize sugars and acidify juice. Strain *L. plantarum* JS052 metabolized fructose and glucose to the greatest extent. Moreover, both *L. plantarum* JS052 and MIX B were the most effective in lowering the pH

of the product, which was also confirmed by the titratable acidity test. It was found that depending on the strain used, both in the form of monoculture and bacterial consortium, the content of selected biologically active compounds can be shaped, which can be presented as follows:

- If the aim is to obtain fermented kale juice rich in B vitamins (in particular vitamin B_2 and B_6), strain *L. sakei* JS032 should be used.
- In order to obtain a product with a high content of phenolic compounds, fermentation should be carried out with *L. plantarum* strain JS052.
- High carotenoid content can be obtained by using *L. sakei* JS032 and MIX A.
- The glucosinolate content can also be modified by the metabolism of the bacteria tested, so the best product performance in terms of glucosinolate content can be obtained after fermentation with mixed cultures (both MIX A and MIX B).

Also, the high antimicrobial activity contributes to the health-promoting properties of the fermented kale juice. In this respect, fermentation with the *L. plantarum* strain JS052 enabled the best performance against all tested indicator microorganisms. Importantly, all tested variants were characterized by sufficiently high growth, and especially the *L. plantarum* JS052 variant showed the best cell count and viability. This demonstrates their technological usefulness, as well as their potential probiotic properties, due to their possible efficient colonization of the intestinal microbiota.

*Therefore, the **fourth hypothesis,** that the kale microbiota is a source of lactic fermentation bacteria useful as starter cultures for modelling selected health-promoting properties of fermented green kale juice, **was verified positively.***

In addition, based on studies conducted during the product development stage, it was found that controlled lactic fermentation contributed to significant changes in the content of various compounds. An increase in the content of B vitamins was observed (in the fermented juice with *L. sakei* JS032 and MIX A culture - pyridoxine and riboflavin, and in the fermented juice with *L. plantarum* JS052 and MIX B - pyridoxine), an increase in the total content of phenolic compounds and a higher content of 9-cis lutein for all tested variants. At the same time, the antioxidant activity remained the same as in the fresh juice, as well as the levels of ferulic acid and other hydroxycinnamic acids remained unchanged for all prototypes. In contrast, the content of individual compounds remained the same depending on the bacterial culture used. The use of MIX A contributed to maintaining the levels of synapic acid, kempherol, all-trans lutein, zeaxanthin and sinigrin. On the other hand, fermentation with *L. plantarum* strain JS052 maintained the levels of quercetin, kempherol and sinigrin also remained unchanged. In the case of using the MIX B variant, the content of the following compounds did not change: synaptic acid, progroitin and sinigrin. The use of *L. sakei* JS032 contributed to maintaining all-trans lutein at the same level as in fresh juice. However, a reduction in individual bioactive

compounds such as caffeylquinic acid, caffeic acid, neoxanthin, violaxanthin, 13-cis lutein, β-carotene, β - cryptoxanthin and indole glucosinolates was observed for all juice prototypes. Also, the level of aliphatic glucosinolates for most compounds decreased in all tested variants. As mentioned earlier, the changes in the content of the studied compounds were strongly related to the metabolism of the starter culture used.

> ***The fifth hypothesis*** *- controlled fermentation of kale juice allows for a higher content of selected bioactive components compared to fresh juice and juice subjected to spontaneous fermentation -* ***was positively verified for*** *an increase in the content of B group vitamins and a higher content of 9-cis-lutein for all tested variants. On the other hand, the* ***negative*** *verification* ***included*** *antioxidant properties, total phenolic compounds content, for indolic glucosinolates, part of phenolic compounds, carotenoids and part of aliphatic glucosinolates.*

The whole research process presented in this dissertation has clearly shown that the design and development of a new food product, on the example of fermented food with health-promoting properties, is a complex process. The health-promoting properties that consumers often expect are fundamentally influenced by the starter cultures used, whose properties are strain-dependent. Therefore, in order to offer an innovative and health-promoting product, consumer research and multifaceted laboratory analyses must be carried out to ensure its comprehensive characterization and to identify potential developments based on strain-dependent metabolic pathways.

It is worth noting that kale juice subjected to controlled fermentation should be considered as an innovative product with functional properties, mainly also due to the presence of beneficial microorganisms with probiotic potential and the maintenance of a high content of most biologically active compounds. Thus, all these health-promoting properties increase its commercial and nutritional value for potential customers interested in non-milk products with probiotic potential.

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