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MICROBIOLOGICAL ANALYSIS OF SAUERKRAUT IN THE PROCESS OF ITS FERMENTATION ACCORDING TO THE TRADITIONAL AND MODERNIZED TECHNOLOGIES

***Aim.** The aim of this work is detection of the differences in the quantitative and qualitative content of the microorganisms involved in fermentation of traditional Ukrainian food – sauerkraut (cabbage) under various conditions of its production. **Methods.** Microbiological methods for the isolation (cultivation on selective chromogenic medium) and identification of isolated microorganisms: using the semi-automatic biochemical test systems and laser desorption technique (MALDI). **Results.** The changes in qualitative and quantitative composition of microorganisms in tested sauerkraut samples taken at different stages of fermentation process by the traditional local (original) recipe and produced by upgraded modern technology have been revealed. In particular, the strains of *Lactobacillus delbrueckii* were isolated from all tested samples of fermented product, while the strain of *L. casei* was isolated only from sauerkraut samples made according to the original recipe. In these same samples, as opposed to those that were manufactured by industrial technology, the number of isolated strains of enterococci was insignificant. **Conclusion.** The results obtained confirmed the significant differences in qualitative and quantitative content of isolated microorganisms isolated from tested sauerkraut samples depending of methodology of its fermentation. The important differences in the composition of microorganisms associations at the beginning and at the end of sauerkraut fermentation have also been detected and defined.*

Key words: traditional food, sauerkraut, original recipe, fermentation, microbial composition.

Introduction

The biochemical changes connected to microorganisms' content that occur during fermentation, are an indicator of the final product quality. This was confirmed by our earlier analysis of chemical content of the main components therefore included in the national composition databases [1].

The study of food products of plant origin, and especially fermentation, is extremely important today, since they are the source of a variety of probiotic and prebiotic substances [2]. Isolated key beneficial microorganisms from fermented cabbage when made traditionally is of great interest and requires further examination



since it is important for sustainable obtaining a qualitative and safe product, and furthermore it might be useful as probiotic base (starter) in the manufacture of modern functional food for personalized implementation. The technological processing of food is crucial in ensuring and maintaining the ingredient's useful properties. Fermentation makes food more digestible, because during the fermentation process foods are not only enriched with biologically active compounds (BAC) but also with microorganisms that enter into the gastrointestinal tract (GIT) with the food [3]. Because local traditional recipes vary, fermentation of plant products also varies and this in turn encourages the production of valuably unique combinations of products. On the other hand, such preparation procedures ensure the availability of nutrients, vitamins, minerals and unique strains of microorganisms. It is proved that lactic acid, which is formed during fermentation, combined with live probiotic microorganisms inhibits the development of opportunistic microorganisms in the gut and normalizes the composition of intestinal microbiota [4, 5]. There is also some evidence of the anti-tumor properties of sauerkraut [6, 7].

Due to the health benefits provided, it is important to use traditional foods manufactured by the original technology in view of the available data of epigenetic studies that show of human microbiota's changes occurred via DNA methylation dependent of eating habits. Our preliminary research that first performed chemical analysis of a number of traditional and also fermented foods made according to the original recipes reported the data for the creation of the first national food composition database [2].

In this paper, we aim to demonstrate that qualitative and quantitative content of microorganisms of the fermented product is depending on the stage and define by fermentation technology.

Materials and methods

In vitro and *in situ* microbiological assessment of the sauerkraut samples was conducted. For isolation and next cultivation of microorganisms – MRS Agar, Bifidobacterium Broth, URI select Agar, Perfringens Agar (OPSP), Orange Serum Agar (HiMedia, India), Dehydrated Culture Media Brilliance™ Candida Agar (formerly Oxoid Chromogenic Candida Agar (OCCA), USA) and nutrient media, Blood culture Medium had been used. Initial diagnosis of genera and species diagnosis of the microbial isolates was conducted using selective chromogenic media (CHROMagar™, USA).

The identification of bacterial species was carried out using substantiated methods; modified algorithms and semi-automatic biochemical test systems ENTERO-test 24 and ANAERO-test 23 (Erba Lachema, Brno, CZ). For confirming (specified) identification, mass spectrometry (MALDI) was used. All Lactobacillus strains isolated from sauerkraut's samples and other fermented traditional for Black Sea region countries' foods were tested for their sensitivity to antibiotics in order to identify their marker characteristics, and their antimicrobial and immunomodulatory properties was also studied [8].

Sauerkraut' samples were taken under a sampling protocol described elsewhere [2] and in accordance with national standard.

To compare microbial and organoleptic features of sauerkraut produced



by the original technology (v. Bedevlya, Tyachiv district) with similar fermented products the samples obtained from city supermarkets (“Dastor” and “Sil’po”) and homemade (Uzhhorod) were studied.

Pro- and anti-microbial properties of sauerkraut liquid soluble substance (juice) were determined at 24; 48; 72 hours. The juice of fermented cabbage was firstly passed through a filter for its sterilisation (with 44 µm pores (BD Falcon, USA)) and ability to grow of chosen bacterial strains the representatives of commensal, beneficial and detrimental microbial groups was studied in vitro and detected in counted of colony forming units (CFU/ml).

Among the selected for this study bacterial strains were isolated earlier clinical cultures: agents of nosocomial infections, opportunistic pathogens (*Staphylococcus aureus* and *Enterobacter cloacea*) and pathogenic bacteria caused gastro-intestinal disorders (*Salmonella enterica* and *Shigella dysenteriae*), and also commensal gut microorganisms (*Escherichia coli* 058, *E. coli* (Schaedler), *Enterococcus faecalis* and *Morganella morganii*) as well as other beneficial microbiota representatives (*Lactobacillus acidophilus*, *Bacillus subtilis* 8130, *Bacillus subtilis* 090). All the strains are belonged to our Centre’ authors’ collection microbial cultures.

All experiments were performed in triplicate and data were processed using the software Origin 8.0.

Results and Discussion

Traditional original technology of homemade sauerkraut (v. Bedevlya, Tyachiv district) is unique and historically documented, and therefore corresponds to the definition of “traditional food”. The specific conditions of its manufacture – in particular, are in using the “centenarian” oak barrels containing original microbial starters on its surface. The product is prepared by traditional technology by aging it for 4 days in a warm place (t=24-28 °C). After 5-6 days, cabbage is taken from the barrel, rearranged into a glass container with a lid to stop fermentation. Other methods of digestion differ primarily by using conventional removable tanks for fermentation.

In the first days of fermentation *Candida dubliniensis*, *C. famata*, *Enterococcus faecium*, *Cryptococcus humicola*, *Lactobacillus casei*, in amount of 10⁴ and 10⁵ CFU/ml, respectively had been detected and isolated. Thus, we can assume that these bacterial species were in the original microbial starting cultures that initiated the fermentation process. No significant changes in the microorganisms’ species had been observed, while their total amount increased on the 3rd day, and particularly for *E. faecium* up to 10⁸ CFU/ml. On the 11th day two strains of *Candida* – *C. dubliniensis*, *C. famata* were isolated and *L. casei* had also been detected at 10⁷ CFU/ml, while *C. humicola* was completely replaced by *Bifidobacterium dentium* –which were at 10⁸ CFU/ml. According to the genetic sequencing this last mention here strain finally had been attributed to *L. plantarum* (by the similarity to *L. plantarum* JCM 1149 strain, RDP 1.000).

After the fermentation process all the other sauerkraut samples made by urban technology and obtained from supermarkets (“Silpo” and “Dastor”) were analyzed. No significant differences in the number of bifidobacteria (>10⁶) had been found. Lactobacilli strains belong to various species and distinguished by their source of isolation.



Namely, from supermarkets samples of sauerkraut the strains of *L. delbrueckii* were isolated in an amount of 5×10^6 CFU/ml while from the samples made by urban technology the strains of *L. casei* was found to be at 10^6 CFU/ml. Enterococci were found only in supermarket samples at 10^5 CFU/ml. These data particularly have been reported earlier with the other results of the microbial content of other fermented products from Black Sea region countries within BaSeFood project [9, 10]. The obtained results are summed up in Table 1, indicating a significant difference in the species composition of microorganisms isolated from different samples of studied sauerkraut.

Table 1

Microbial strains isolated from sauerkraut in the previous qualitative analysis

<p>Sauerkraut: isolated strains of microorganisms from all the tested samples Lactobacillaceae / <i>Lactobacillus</i> / <i>Lactobacillus plantarum</i>, <i>L. curvatus</i>, <i>L. paraplantarum</i>, <i>L. coryniformis</i>, <i>L. brevis</i>, <i>L. lactis</i> subsp. <i>lactis</i> Leuconostocaceae / <i>Leuconostoc</i> / <i>Leuconostoc fallax</i>, <i>L. citreum</i>, <i>L. argentinum</i>, <i>L. mesenteroides</i> Pediococcus <i>Pediococcus pentosaceus</i> Clostridiaceae/<i>Clostridium</i> / <i>Clostridium buturicum</i> Candida <i>Candida dubliniensis</i>, <i>C. famata</i></p>
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Selected microbial species, changes, qualitative assay:

Sample, NO	Candida	Saccharomyces	Escherichia	Staphylococcus	Lactobacillus	Enterococcus	Proteus	Bacillus	Pseudomonas
1	+	+	+	+	+	+	+	—	++
2	+	+	+	+	—	—	+++	—	+++
3	—	—	++	+++	—	+	++	—	++
4	++	+	+	+	+	—	—	—	—
5	+++	+	+	—	++	++	—	—	—
6	+++	+	—	—	+++	+	—	+	—

Note: 1–3 – probes selected in supermarkets, 4 – sauerkraut of home-made fermentation, 5–6 – probes selected in v. Bedevlya (original traditional preparation recipe of sauerkraut, research in situ), + (++, +++) – means the presence of isolated microorganism and its relative allocation frequency, “—” – its absence in test samples.

Figure 1 shows the results of stage dependent microbiological assessment of sauerkraut (in dynamics) selected in v. Bedevlya (original traditional preparation recipe of sauerkraut, research *in situ*).

As seen in Figure 1, on the first day of assay the strains of *L. casei*, *C. humicola*, *E. faecium*, *C. famata*, *C. dubliniensis* within 10^4 – 10^5 CFU/ml have been isolated. After the 3rd day the same species of microorganisms was allocated but in increased number – up to 10^7 – 10^8 CFU/ml. On the 11th day of study, we



have shown the reduction of *E. faecium* down to 10^6 CFU/ml, growth inhibition of *C. humicola*, and isolated in high amount (10^7 – 10^8 CFU/ml) *B. dentium*, when the number of *C. dubliniensis* and *C. famata* has not significantly changed. On the 20th day there were isolated mainly *L. delbrueckii* (10^7 CFU/ml), *L. casei* (10^6 CFU/ml) and *B. dentium* (10^7 – 10^8 CFU/ml).

We found also a significant difference in the composition of microbial associations at the beginning and at the end of fermentation.

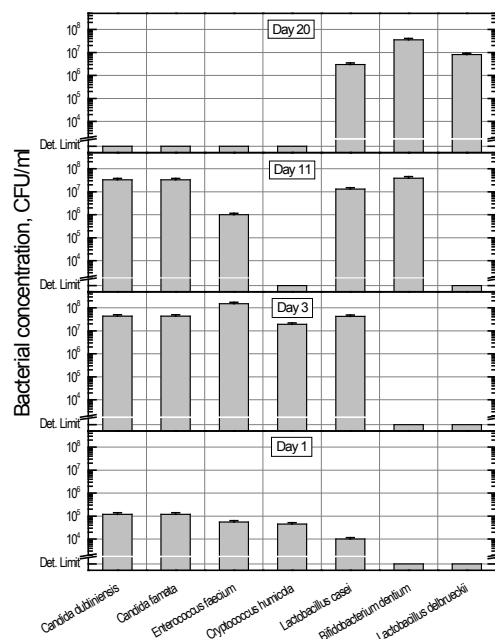


Fig. 1. Microorganisms isolated in situ in the village Bedevlya (Tyachiv district, Transcarpathian region) from fermented foods (homemade sauerkraut)

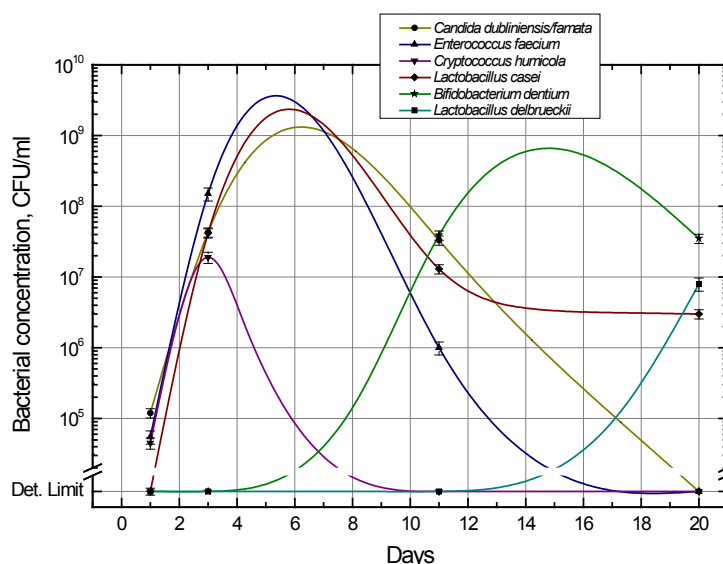


Fig. 2. Regularities of cultivation of microbial cultures' cocktail during fermentation of homemade sauerkraut obtained by traditional technology



Table 2

Results of organoleptic evaluation of studied samples

Indicator	Standard data	Results of the studied samples of sauerkraut		
		from supermarket	local "home" cooking technology	rural traditional homemade original technology
Consistence	juicy, tight, crisp or moderately tight and crisp for second grade	juicy, tight, moderately crisp	juicy, tight, crisp	juicy, tight, crisp
Appearance	cabbage evenly shredded, no wider than 5 mm, vegetable ingredients, spices are evenly distributed in the cabbage.	Inherent to this type of product, meets the standard	Inherent to this type of product, meets the standard	Inherent to this type of product, meets the standard
Taste	sour-salty, pleasurable, without bitterness. For second grade more sharply expressed sour-salty taste	sour-salty, pleasurable, without bitterness	sour-salty, pleasurable, without bitterness	sour-salty, pleasurable, without bitterness
Smell	aromatic, typical for sauerkraut. In the cabbage with herbs and spices clearly felt aroma of added spices. Juice has cabbage flavour	aromatic, typical for sauerkraut, clearly felt aroma of added spices; juice has flavor of spices	aromatic, typical for sauerkraut, clearly felt aroma of added spices; juice has cabbage flavor.	aromatic, typical for sauerkraut, clearly felt aroma of added spices; juice has cabbage flavor
Color	light straw with a yellowish tinge. In the cabbage with herbs and spices can be shades depending on the color of added condiments and spices. For second grade cabbage - light yellow with a greenish tinge	light yellow with a greenish tinge. Expressed shades of spices	light straw with a yellowish tinge. Expressed shades of spices	light straw with a yellowish tinge. Expressed shades of spices

Table 3

Results of co-cultivation of sauerkraut juice with tested microorganisms *in vitro* studies

№	Tested microorganisms, author's strains	Number of microorganisms, CFU/ml		
		24 hours	48 hours	72 hours
1	<i>B. subtilis</i> 090	$(9\pm 0,4)\times 10^6$	$(7\pm 0,1)\times 10^6$	$(5,5\pm 0,3)\times 10^6$
2	<i>B. subtilis</i> 8130	107	$(5\pm 0,2)\times 10^7$	$(9\pm 0,1)\times 10^8$
3	<i>E. coli</i> (Schaedler)	$(5\pm 0,2)\times 10^7$	0	0
4	<i>E. coli</i> 058	$(7\pm 0,3)\times 10^6$	0	0
5	<i>S. enterica</i>	$(1,5\pm 0,1)\times 10^8$	106	0
6	<i>S. dysenteriae</i>	108	$(5\pm 0,3)\times 10^5$	0
7	<i>E. cloacae</i>	$(1,5\pm 0,2)\times 10^8$	106	0
8	<i>S. aureus</i>	$(6\pm 0,3)\times 10^8$	$(2\pm 0,1)\times 10^4$	0
9	<i>M. morgani</i>	108	$(3\pm 0,2)\times 10^4$	0
10	<i>L. acidophilus</i>	$(1,2\pm 0,1)\times 10^7$	$(3,5\pm 0,4)\times 10^8$	$(4\pm 0,1)\times 10^8$
11	<i>E. faecalis</i>	$(4\pm 0,3)\times 10^7$	103	0
12	<i>B. dentium</i> (<i>L. plantarum</i>)	$(5\pm 0,5)\times 10^7$	108	$(2\pm 0,1)\times 10^8$

Figure 2 shows the particularity and growing specificity of each single strain's characteristics in certain microbial co-cultures' cocktail via putting it into the general regularities of fermentation process. The strain of *E. faecium* was found in sufficient quantities on the 4th day, when the sauerkraut was ready for use. On the 11th day this strain was also isolated, but in smaller quantities. Opposing increasing functions were shown for strain *L. delbrueckii*, which was discovered only on the 20th day (10^7 CFU/ml). All isolated cultures after a detailed study, detection of strain-specific markers and certification procedure were deposited in the Collection of Microbial Cultures of Institute of Microbiology and Virology of National Academy of Science of Ukraine (IMV NASU) for further use in the preparation of traditional dishes of personalized application [11]. In comparison by organoleptic characteristics all the studied samples of sauerkraut meet the requirements of the national standard [12]. Comparison results shown in Table 2.

Earlier we reported of pro- and anti-microbial properties of isolated microorganisms [8] and content of biologically active compounds of sauerkraut produced by the original recipe [1]. Here we present the results of the studied effect of sauerkraut juice containing microbial starters' metabolites on tested microorganisms which belongs to various groups of microorganisms – commensal, beneficial, potentially pathogenic and detrimental bacterial strains (Table 3).

As it can be seen from Table 3, sauerkraut juice is characterized by antimicrobial activity concerning the commensal strains of microorganisms *E. coli* 058, *E. coli* (Schaedler), *E. faecalis* and *M. morgani* and also against the agents of human nosocomial – *S. aureus*, *E. cloacae* and gastro-intestinal – *S. enterica* and *S. dysenteriae* infections. At the same time, we have observed the neutral influence of the sauerkraut juice on spore forming bacteria and its stimulating effect on strains *L. acidophilus* and *B. dentium* (*L. plantarum*).



Conclusions

As a result of performed in dynamics microbiological study of Ukrainian fermented product (sauerkraut), produced according to the traditional recipe, original strains of microorganisms that initiate and trigger fermentation process, namely *C. dubliniensis*, *C. famata*, *L. casei*, *L. delbrueckii*, *E. faecium*, *C. humicola*, *B. dentium* (*L. plantarum*) have been isolated and identified.

The key microorganisms defying and ensuring the quality of fermented cabbage as one of the prioritized national foods have been also revealed.

The differences in species and quantitative composition of microorganisms in the beginning, during and at the end of fermentation in dependence of manufacturing technology of the product have been carefully monitored, detected and interpreted.

By microbiological and organoleptic indexes all the studied sauerkraut samples made by different technologies, in general corresponded the general requirements for products of this type, but differed significantly in quality, consistency, smell and odor.

The best studied food product was sauerkraut made by the original traditional technology (Bedevlya village, Tyachiv district). We have studied pro- and antimicrobial properties of sauerkraut juice, showed its ability in vitro to inhibit growth of commensal (*E. coli* 058, *E. coli* (Schaeidler), *E. faecalis*, *M. morgani*), potentially pathogenic (*S. aureus*, *E. cloacae*), and pathogenic microorganisms (*S. enterica*, *S. dysenteriae*) and stimulate the growth of lactobacilli (*L. acidophilus*, *B. dentinum* (*L. plantarum*)) while not exerting any detectable effect on spore forming aerobic bacteria (*B. subtilis*, strains 090 and 8130).

Isolated from fermented in local conditions by original traditional recipe sauerkraut key beneficial microorganisms are in high interest for its further examination and next application for the sustainable obtaining a qualitative and safe product(s), and on the other hand for their potential usage as probiotic strains (starters) in the manufacture of modern functional food of personalized implementation. Isolated Lactobacillus strains are deposited in the Depository of microbial cultures of IMV NASU for further use in the preparation of traditional dishes of personalized application.

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МІКРОБІОЛОГІЧНИЙ АНАЛІЗ КВАШЕНОЇ КАПУСТИ ПРИ ФЕРМЕНТАЦІЇ ЗА ТРАДИЦІЙНОЮ ТА МОДЕРНІЗОВАНОЮ ТЕХНОЛОГІЯМИ

Реферат

Мета. Метою даної роботи є дослідження відмінностей у кількісному і якісному складі мікроорганізмів, що беруть участь у процесі ферментації української традиційної страви – квашеної капусти за різних умов її виготовлення. **Методи.** Мікробіологічні методи досліджень для виділення



(культивування на селекційних хромогенних середовищах) та ідентифікації ізольованих мікроорганізмів з використанням напіваавтоматичних біохімічних тест-систем і методу лазерної десорбції (MALDI). **Результати.** Встановлено зміни мікробіологічного складу зразків капусти квашеної, відібраних на різних стадіях бродіння в процесі її ферментації за традиційною локальною і модернізованою сучасною технологіями. Зокрема штами *Lactobacillus delbrueckii* були ізольовані з усіх протестованих зразків ферментованого продукту, тоді як штам *L. casei* виділяли лише із зразку квашеної капусти, виготовленої за оригінальною рецептурою. У цих же взірцях на противагу тим, які були виготовлені за промисловою технологією, кількість ізольованих штамів ентерококів була незначною. **Висновки.** Отримані результати свідчать про суттєві відмінності у якісному і кількісному складі ізольованих мікроорганізмів у досліджених взірцях в залежності від методології бродіння квашеної капусти. Встановлено наявність істотної різниці у складі асоціації мікроорганізмів на початку і в кінці бродіння квашеної капусти.

Ключові слова: традиційні страви, квашена капуста, оригінальна рецептура, процес ферментації, мікробний склад.

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МИКРОБИОЛОГИЧЕСКИЙ АНАЛИЗ КАПУСТЫ БЕЛОКАЧАННОЙ (КВАШЕНОЙ), ИЗГОТОВЛЕННОЙ СОГЛАСНО ТРАДИЦИОННОЙ И МОДЕРНИЗИРОВАННОЙ ТЕХНОЛОГИИ БРОЖЕНИЯ

Реферат

Цель. Целью данной работы является тщательное исследование различий в количественном и качественном составе микроорганизмов, принимающих участие в процессе ферментации украинского традиционного блюда – квашеной капусты (белокочанной) при различных условиях ее изготовления. **Методы.** Микробиологические методы исследований для выделения (культивирования на селекционных хромогенных средах) и идентификации изолированных микроорганизмов: с использованием полуавтоматических биохимических тест-систем и метода лазерной десорбции (MALDI). **Результаты.** Определены изменения микробиологического состава образцов квашеной капусты, отобранных на различных стадиях брожения в процессе ее ферментации по традиционной локальной (оригинальной) рецептуре и изготовленной по модернизированной современной технологии. В частности штаммы *Lactobacillus delbrueckii* были изолированы из всех протестированных образцов ферментированного продукта, тогда как штамм *L. casei* выделяли только с образца квашеной капусты, изготовленной по оригинальной рецептуре. В этих же образцах в противовес тем, которые были изготовлены по промышленной технологии, количество изолированных штаммов энтерококков была незначительной. **Выводы.** Полученные результаты свидетельствуют о существенных различиях в качественном и



количественном составе изолированных микроорганизмов в исследованных образцах в зависимости от методологии брожения квашеной капусты. Показано наличие существенной разницы в составе ассоциаций микроорганизмов в начале и в конце брожения квашеной капусты.

Ключевые слова: традиционные блюда, квашеная капуста, оригинальная рецептура, процесс ферментации, микробный состав.

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