

Effect of drying process on survival of environmental starter culture and nutritional value of fermented rapeseed meal as feed for pigs*

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Summary

Since Poland is one of the largest producers of rapeseed, the products of its processing – oil cake and rapeseed meal – could be promising alternatives in animal nutrition and, in the future, could also be used as food additives for humans. Fermentation, of these post-waste raw materials, eliminates the bitter taste and enriches the ferment with nutrients. Due to the fact that fermented rapeseed meal has a high water activity, it is possible to develop virtually all groups of microorganisms in it. Therefore, it is advisable to use a fixation method that will ensure the microbiological stability of the fermented product. The purpose of this study was to develop a starter culture for the fermentation of rapeseed meal and to investigate the effect of the drying process of fermented rapeseed meal on the survival rate of the starter culture. Fermented rapeseed meal was dried at 48°C for 33 h. After 30 days of storage of the dried meal, the survival rate of the starter culture was checked. Since the fermented rapeseed meal was meant to be used as feed for pigs, a low-temperature drying process was used in the study. Drying the fermented rapeseed meal made it possible to stabilize the product and allowed it to be stored safely, without signs of mold, for 30 days. The drying temperature of 48°C did not negatively affect the biological value of the nutrients or the survival of the starter culture in dried rapeseed meal after rehydration.

Keywords: rapeseed meal, vegetable protein, fermentation, starter culture

Rapeseed is the second largest source of vegetable protein with a good amino acid profile in the world. Rapeseed meal is one of the products of rapeseed oil extraction. It contains about 35-40% protein with a well-balanced amino acid composition (36). The protein content is influenced by the type of rapeseed, as well as its growing environment and fiber content. Meal contains several minerals, such as calcium, magnesium, and copper, a number of vitamins, and other bioactive compounds, such as phenols, which increase its nutritional value. However, the use of rapeseed

meal in pig nutrition is limited due to anti-nutritional factors, mainly glucosinolates, toxic substances, and poor palatability (8, 12, 36, 37). Undecomposed glucosinolates are not harmful, but when hydrolyzed by the enzyme myrosinase, found in rapeseed meal, or by bacterial enzymes, they produce compounds (isothiocyanates, vinylloxazolidinethiones, and nitriles) that disrupt thyroid, liver, and kidney function. Myrosinase is activated when the structure of the seed is disturbed, but loses activity when heated. In the “cold” extrusion process, the temperature of the pomace can reach 80°C, or higher when extrusion is used. One method of improving the nutritional value and flavor of rapeseed meal is fermentation (8, 12, 36, 37).

Microorganisms are an integral part of all ecological niches, colonizing even the most extreme environ-

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ments. It is mainly metabolic activity that determines whether microorganisms have a positive or negative impact on the environment. The beneficial effect of microbes is utilized in various biotechnological processes, including the production of food, feed, medicines (including antibiotics), vaccines, and plant protection products. Among various environmental microorganisms of biotechnological importance, special attention is paid to bacteria that are used in many industries. The most important group are lactic acid bacteria (LAB), used in the agri-food industry. Thanks to their fermentation abilities, they have numerous technological applications (2, 20). They are used as starter cultures to produce dairy products: yogurts, fermented drinks, and cheeses, for acidifying vegetables, fruit, and feed, or for producing bread, meat products, pastes, sauces, and wine. The presence of LAB improves the taste of products and extends their shelf life, as well as provides valuable bioactive metabolite ingredients, which are often postbiotics (2, 20). Lactic acid bacteria, which naturally colonize the body of mammals, occur in the oral cavity, digestive tract, and reproductive tract (10, 20). Thanks to their activities (such as lowering pH, producing bacteriostatic and bactericidal substances, restoring the balance of T helper 1 (Th1): T helper 2 (Th2) lymphocytes, stimulating the activity of macrophages), LAB inhibit the growth of pathogenic microorganisms, conditioning the homeostasis of organisms and having a positive effect on immunity (10). Therefore, eating fermented food is highly beneficial for the health of humans and animals (3). Some of the LAB strains, meeting restrictive criteria and health claims, can be classified as probiotics and successfully used in the treatment of many diseases, such as lactose intolerance, inflammatory bowel disease (Crohn's disease, ulcerative colitis, non-specific colitis, eosinophilic or collagenous colitis), Behcet's disease, microbiota disorders after antibiotic treatments, allergies, celiac disease, cancer, and urogenital tract infections (10, 20).

Depending on the type of fermentation, the main metabolites of LAB are organic acids (lactic acid, acetic acid, propionic acid), diacetyl, carbon dioxide, bacteriocins, glycerol, and ethanol (10, 20). The LAB most frequently used in fermentation processes are *Lactococcus* spp., *Streptococcus* spp., and *Lactobacillus* spp. (10).

In addition to lactic bacteria, yeasts are also commonly used in the agro-food industry. Due to their cosmopolitan nature, they are found in large numbers in many environments, functioning both in the presence of oxygen and under anaerobic conditions. They have enormous biotechnological potential due to their unique physiological and biochemical properties. They are used in the baking, dairy, distilling, brewing, and wine industries. In the process of alcoholic fermentation, they produce flavorful, aromatic substances giving products unique organoleptic qualities. They can be

used in the production of dietary supplements, valuable food additives, functional foods, or medicines. They also have a high nutritional value, being a rich source of protein, vitamins, especially B vitamins, minerals, beta-glucans, mannans, and bioactive substances (14).

The fermentative capabilities of LAB and yeast could be an interesting tool for producing plant-based alternatives to meat. The growing public awareness of the health and environmental benefits of plant protein is causing major changes in the protein market (3). The fermentation process reduces the content of antinutrients and allergens, while increasing the amount of essential micronutrients, health-promoting bioactive compounds, as well as crude and soluble protein. During the fermentation process, various types of enzymes are produced: glucosidases, cellulases, and amylases, which participate in the hydrolysis of toxic substances. Fermentation produces vitamins and fatty acids of low molecular weight (3, 5, 6, 8, 11, 13). Due to the fact fermented rapeseed meal has high water activity, an additional preservation method is required to ensure longer microbiological stability of the ferment. The results presented here are part of research to develop fermented rapeseed meal for monogastric animals. In the present study a low-temperature drying process was employed, which can be successfully used on farms. Dried fermented rapeseed meal is easy to store, transport, and dose into feeding systems.

In view of the possible use of rapeseed meal as animal feed, research was carried out to ferment rapeseed meal with a selected culture of environmental microorganisms under controlled conditions and to investigate the effect of low-temperature drying of fermented rapeseed meal on the survival of environmental starter microorganisms and the nutritional value of the fermented product.

Material and methods

Biological material. Lactic acid bacteria and yeasts have been isolated from natural biological materials (silages and baking leavens) and identified. To develop the starter culture, strains with the best biochemical characteristics, capable of fermenting rapeseed meal, were selected. Lactic acid bacteria were isolated and identified with appropriate API media and tests: API® 50 CH, and API® 50 CHL media (bioMérieux, France). Approximately 50 isolates of bacteria and yeasts were isolated, mainly belonging to the following species: *Latilactobacillus curvatus*, *Leuconostoc mesenteroides*, *Pediococcus damnosus*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Lactobacillus acidophilus*, *Candida glabrata*, and *Saccharomyces cerevisiae*.

Starting culture. The starter culture for the rapeseed meal fermentation process consisted of 4 microorganisms: *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, *Leuconostoc mesenteroides*, and *Saccharomyces cerevisiae*.

Preparation of inoculum. The starter culture was added to wholemeal flour (wheat and rye) while mixing with lukewarm water. It was thoroughly distributed until a uniform biomass was obtained, in which the initial density of

microorganisms was 5 log CFU/g of lactic acid bacteria and 6 log CFU/g of yeast. The biomass was incubated at temperatures of 21-22°C and humidity in the range of 20-28% for 72 hours.

Fermentation of rapeseed meal. The 72-hour inoculum, in which the number of LAB and yeast was 6.3 ± 0.06 log CFU/g and 7.8 ± 0.03 log CFU/g, respectively, was added in an amount of 20% (V/V) to rapeseed meal. The acidification process was carried out at temperatures of 21-22°C and humidity in the range of 20-28% for up to 96 hours, controlling the physicochemical and microbiological parameters.

Drying. After acidification, the rapeseed meal was dried in a laboratory dryer VD53 (BINDER GmbH, Germany) without air circulation at a temperature of $48 \pm 2^\circ\text{C}$ for 33 h, with a material layer thickness of approximately 2 cm. After drying, the meal was packed in sterile polyethylene bags (Nasco Whirl-Pak, USA) and closed with a tight, hermetic metal clamping tape. The dried meal was stored at a temperature of 21-22°C and humidity in the range of 20-28% for 30 days.

Microbiological determinations. Microbiological tests for the number of mesophilic lactic acid bacteria, molds, and yeasts were carried out in accordance with applicable standards, in acidified meal, dried meal, and rehydrated meal. The following standards were used to determine these microbiological parameters:

- determination of the number of mesophilic lactic acid bacteria with MRS medium (Merck, Germany) $30^\circ\text{C} \pm 1^\circ\text{C}/72$ h (31);
- determination of molds and yeasts with chloramphenicol media (Merck, Germany) $25^\circ\text{C} \pm 1^\circ\text{C}/5$ days (33).

Water activity. Water activity (a_w) was determined with an AquaLab 4TE device (MeterGroup, USA) at a temperature of $25^\circ \pm 0.2^\circ\text{C}$ by filling disposable measuring containers and placing them in the device chamber. After the measurement chamber had been tightly closed, a few minutes were allowed for the equilibrium state to be reached. The relative humidity of the air in the chamber in a state of thermodynamic equilibrium corresponded to the air humidity in the sample.

Measurement of pH. The pH was measured using an ELMETRON CX505 pH meter (ELMETRON, Poland) with an EPS-1 electrode. The measurement was made by immersing the electrode in the sample, and after determining electrode readings, the result was read with an accuracy of 0.01 (27).

Acidity. A sample weighing 2 g was mixed with 50 cm³ of distilled water (temp. 20°C). Acidity was measured using an AT1000 titrator equipped with a glass composite electrode Intellical PHC805. After determining electrode readings, the result was read with an accuracy of 0.01 (30).

All measurements were performed in triplicate.

Temperature monitoring. The temperature and humidity of the acidification and storage of the product were controlled by a continuous monitoring system installed in the laboratory with calibrated temperature and humidity recorders from TandD Series RTR500B (TandD, Japan).

Energy value. The energy value was calculated according to the content of components in unfermented rapeseed meal, acidified rapeseed meal, and dried fermented rapeseed

meal. All the values used in the calculations except water were converted to dry weight according to the formula $\text{score} \times 100\%/\text{dry weight}$

Water content. Water content in dry rapeseed meal was determined by the gravimetric method in accordance with the PN-A-74011-1986 standard. Briefly, 5 g of the sample was dried for 60 minutes in a laboratory oven at 130°C (26). The result is reported as dry matter content (29).

The water content of acidified and dried acidified rapeseed meal was determined by the gravimetric method in accordance with the PN-A-82100:1985 standard. Briefly, 5 g of the sample was dried at 105°C to a constant weight. The result is reported as dry matter content (29).

Protein content. Protein content was determined by the Kjeldahl method in accordance with the PN-A-04018:1975/Az3:2002 standard. The total nitrogen content obtained was multiplied by the Kjeldahl conversion factor 6.25 to arrive at the protein content (25).

Fat content. The fat content was determined by the gravimetric method (Soxhlet extraction) according to the PN-A-82100-1985 standard (29).

Dietary fiber content. The fiber content was established by the enzymatic technique in accordance with the brochure for the method AOAC985.29 (1997). One gram of the sample was exposed to an enzyme set consisting of α -amylase, protease, and amyloglucosidase. The individual stages of the study were performed in accordance with a technical brochure for the enzymatic method used (4).

Ash content. The ash content was analyzed by the gravimetric method in accordance with the PN-ISO 2171:2010 standard. The method consisted in incinerating 5 g of the sample in a muffle furnace at a temperature of $900^\circ\text{C} \pm 25^\circ\text{C}$ to a constant mass (32).

Sugar content. The sugar content was determined according to the PN-A-79011-5:1998 standard. The method involved the estimation of reducing sugars based on the amount of sodium thiosulfate solution used for titration of iodine, corresponding to the copper reduced by sugars contained in the sample (28).

Carbohydrate content. The carbohydrate content was calculated based on the content of basic ingredients according to the formula

$$\text{Wog} = 100 - (\text{B} + \text{W} + \text{T} + \text{P})$$

Wog – total carbohydrate content [g/100 g],

B – protein content [g/100 g],

W – water content [g/100 g],

T – fat content [g/100 g],

P – ash content [g/100 g].

Statistics. Microsoft Excel 2016 was used to statistically analyze the test results. All determinations were made in triplicate, and the results were compared using the T test for dependent samples, analyzing differences between the results for fermented rapeseed meal and dried fermented rapeseed meal, and between the results for dried and hydrated rapeseed meal, with $p\text{-value} \leq 0.05$.

Nutrient content results were analyzed by one-way ANOVA. The significance of differences between the average results for rapeseed meal and fermented dried rapeseed meal was calculated using the Bonferroni correction with $p\text{-value} \leq 0.05$.

Results and discussion

Changes in the number of microorganisms added as the starter culture during the fermentation process of rapeseed meal were examined. Physicochemical parameters: pH and acidity (Tab. 1) were also controlled. The number of lactic acid bacteria and the number of yeasts increased with the extension of the fermentation time. At the end of fermentation (96 h) the numbers of LAB and yeasts were 8.46 log CFU/g and 7.82 log CFU/g, respectively. The number of moulds was < 1 log CFU/g. This is because lactic acid strains produce antifungal agents that effectively inhibit the growth of moulds.

During fermentation, acidity increased and pH decreased, amounting to 1.77 SH° and 4.43, respectively, after 96 h.

In the present research, a drying process was used to preserve fermented rapeseed meal. This method of conservation can be successfully used on farms with their own dryers. The meal produced after drying is light, can be stored for a long time without signs of molding,

Tab. 1. Dynamics of the rapeseed meal fermentation process

Parameter	Fermentation time [h]				
	0	24	48	72	96
Lactic acid bacteria number [log CFU/g]	5.2 ± 0.03	6.5 ± 0.02	7.3 ± 0.03	7.9 ± 0.03	8.5 ± 0.02
Yeast number [log CFU/g]	6.4 ± 0.03	7.7 ± 0.01	7.8 ± 0.02	7.9 ± 0.03	7.8 ± 0.02
Mold number [log CFU/g]	< 1.00				
Acidity	0.58 ± 0.06	1.18 ± 0.13	1.37 ± 0.15	1.62 ± 0.17	1.77 ± 0.19
pH	5.26 ± 0.15	4.70 ± 0.15	4.62 ± 0.15	4.52 ± 0.15	4.43 ± 0.15

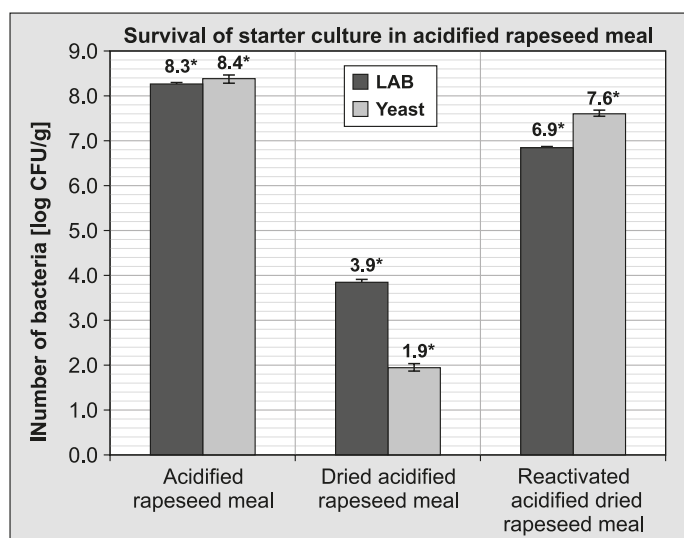


Fig. 1. The influence of the drying and hydration process on the survival of the starter culture in fermented rapeseed meal

Tab. 2. Water activity in fermented rapeseed meal

Rapeseed meal (RM)	Water activity
Fresh, (FRM)	0.9923 ± 0.0012
DRFM	0.7323 ± 0.0021*
DRFM, after 30 days of storage	0.7333 ± 0.0011*
Hydrated, FRM	0.9950 ± 0.0020*

Explanation: * p-value ≤ 0.05

and is easily dosed into the feed system without caking. However, since potentially beneficial lactic acid bacteria (LAB) and yeasts can be inactivated during drying, the effect of this process on the survival of starter microorganisms was examined. The impact of the drying process on the survival of the starter culture is shown in Figure 1.

Before the drying process in fermented rapeseed meal (FRM), the numbers of LAB and yeasts were 8.3 ± 0.02 log CFU/g and 8.4 ± 0.10 log CFU/g, respectively (Fig. 1).

A statistically significant decrease in the number of lactic acid bacteria and yeast was observed after the drying process. The number of LAB and yeast decreased by approximately 4.4 log and 6.5 log, respectively (Fig. 1). At the same time, water activity (a_w) also decreased during the drying process. A statistically significant decrease in a_w (Tab. 2) was observed in dried fermented rapeseed meal (DRFM), from 0.9923 ± 0.0012 to 0.7323 ± 0.0021 . After hydration of fermented rapeseed meal, a statistically significant increase in a_w was observed

to 0.9950 ± 0.0020 (Tab. 2). Thanks to the reduction of water activity, the meal was microbiologically stable for 30 days of storage in room conditions, and the mold growth was limited (< 1.00 log CFU/g).

After hydration of dried rapeseed, there was a statistically significant increase in

the number of lactic acid bacteria by 3 logarithmic orders (Fig. 1). In the case of yeast, the results obtained were analogous to the number of LAB. After hydration, the number of yeasts increased from 1.9 ± 0.03 log CFU/g to 7.6 ± 0.11 log CFU/g. All results were statistically significant. The number of molds in hydrated (FRM) after 30 days of storage was below 1.00 log CFU/g.

Specific nutrients were estimated in rapeseed meal: unfermented (RM), fermented (FRM), and dried after fermentation (DRFM). The tests were carried out in accordance with Polish standards (4, 25, 26, 28, 29, 32), and the results are shown in Tab. 3. Fermentation significantly reduced the amount of dietary fiber.

The protein content ranged from 33.5% (RM) to 31.7% (DRFM), and a statistically significant decrease in the protein content was noted in both FRM and DRFM compared to RM. Statistically significant differences between the three meals were also noted in the content of other nutrients, i.e. protein, ash, sugar, and carbohydrates.

According to the Polish legislation, genetically modified feed, will be prohibited after January 1, 2025, so new substitutes are being sought. Fermented rapeseed meal is a promising alternative to meal obtained from genetically modified soybeans, commonly imported into European countries. Because of the susceptibility of fermented rapeseed meal to rapid microbial deterioration, it is advisable to use a preservation method. Drying at high temperatures (115-145°C) reduces the number

Tab. 3. Nutritional value of rapeseed meals

Nutrients [g/100 g]	Rapeseed meal (RM)	Fermented rapeseed meal (FRM)	Dried fermented rapeseed meal (DFRM)
Protein	33.5 ± 0.1	32.7 ± 0.1*	31.7 ± 0.2*
Fat	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
Water	87.9 ± 0.2	23.6 ± 0.1*	88.5 ± 0.3*
Ash	7.6 ± 0.1	6.7 ± 0.2*	7.5 ± 0.1
Sugar	8.5 ± 0.2	1.8 ± 0.2*	0.6 ± 0.1*
Fiber	39.6 ± 0.2	34.5 ± 0.2*	33.2 ± 0.2*
Carbohydrates	18.8 ± 0.2	19.7 ± 0.2*	23.4 ± 0.3*

Explanation: * statistically significant results at p -value ≤ 0.05 . The values given in the Tab. 3 are average values converted into dry weight, taking into account the standard deviation.

of pathogenic and health-promoting microorganisms (9, 17). Therefore, it is important to select appropriate process parameters: temperature, time, water activity, etc. High temperature may inactivate bacteria added as starter culture, denature proteins, and inactivate bioactive ingredients, thus impairing the nutritional value (12, 15, 24, 34). In research conducted by Krasucki et al. 2002 (15), the impact of different temperatures on the content of basic components of rapeseed seeds was investigated. Rapeseed seeds were dried at temperatures of 80, 120, 150, and 180°C. No changes were recorded in the content of the seed components at the lowest temperatures: 80 and 120°C, but changes did occur at higher temperatures. Significant changes in lipid-protein-carbohydrate structures were observed in seeds exposed to temperatures of 150 and 180°C. Moreover, changes were found in the content of crude fat, crude fiber and its fractions, as well as in the composition of fatty acids (15).

The present research confirmed the benefits of the low-temperature drying process, which did not negatively affect the nutritional value of FRM.

RM protein is an attractive source of bioactive peptides, which can be transformed through enzymatic hydrolysis into peptide fractions with antihypertensive, antioxidant, and antithrombotic properties. The protein content in the dried product remained high, above 30%, thus meeting the need for essential amino acids (7).

Numerous studies have shown that fermentation of RM improves its nutritional characteristics, especially by reducing the content of dietary fibers and glucosinolates (16, 23). The decrease in the fiber content in acidified material results from the presence of fiber-degrading enzymes. The fiber in rapeseed meal is composed mainly of non-starch polysaccharides (NSP), lignin, and non-digestible oligosaccharides, resistant to auto-enzymatic digestion, which can be partly degraded by microbial enzymes originating, for example, from starter culture. After enzymatic hydrolysis, polysaccharides are broken down into smaller molecules such as sugars and uronic monomers. These nutrients can be more easily metabolized by microbiota present in the digestive tract (16). Moreover, the reduced content of dietary fiber in fermented rapeseed meals noted in this

study is beneficial because a high content of dietary fiber is considered as a major factor in poor utilization of protein and amino acids by simple-stomached animals compared to their utilization of protein from soybean meal (38).

Fermentation regulates the balance of intestinal flora, degrades most of the anti-nutritional components, and enriches the taste of acidified feed. By lowering the pH, fermentation further reduces the development of pathogenic microorganisms in the feed. Due to the fermentation process, lactic acid bacteria and yeast multiply and produce lactic acid and acetic acid, which leads to a lower pH. The lower pH of the feed contributes to a lower pH in the stomach of animals. A low stomach pH prevents the proliferation of pathogenic microorganisms (2, 11, 21).

The temperature of 48°C used in the drying process did not negatively affect either the nutrient content or the survival of the starter culture. The dehydration made it possible to obtain a fermented product with reduced water activity, thus extending its durability. After 30 days of storage, the dried rapeseed meal showed no signs of microbiological deterioration. In dried fermented rapeseed meal, a_w significantly decreased. Water activity is a parameter that determines the intensity of chemical, physical, biological, and biochemical processes, as well as the development of microorganisms (34, 35). This parameter has gained importance as an indicator of food safety and microbiological stability. The results are in accordance with other studies. Abdullah et al. (1) showed no mold growth in corn flour with $a_w = 0.808$ stored for 8 months at 25°C. According to data from other authors, a water activity of approximately 0.7 indicates the microbiological stability of dried rapeseed meal and its long shelf life, of up to 2 years (22).

The number of microorganisms in the starter culture was reduced after the drying process as a result of damage from temperature and dehydration. Lactic acid bacteria and yeast, being vegetative forms of microorganisms, are characterized by low thermostability and a high rate of cell death. The mechanism of microbial cell death during drying and storage is still not fully understood. In the drying temperature range of 40-60°C, dehydration and thermal inactivation occur, which is manifested by a high rate of cell death and loss of activity due to denaturation of nucleic acids and proteins, damage to the cytoplasmic membrane, and loss of cell turgor (18, 19). If drying is performed incorrectly, these changes are often irreversible and may cause sublethal or lethal damage to cells. Although these changes depend largely on the individual sensitivity of each strain of microorganisms, equally important are the proper selection of the parameters of the drying process, as well as the medium itself and protective substances (19).

After hydration, the starter culture grew statistically significantly, which confirms that the slow, low-temperature drying process used in this study did not negatively

affect the survival of the starter culture. Furthermore, the LAB and yeast cultures were stable during the 30-day room storage period. The results suggest that rapeseed meal was a good carrier for the starter culture. In order to increase the resistance of microbial cells to temperature and osmotic stress during drying, various protective substances are used (18). Rapeseed meal, derived from the seeds of an oilseed plant, contains not only hydrophilic substances, but also fats: hydrophobic substances that can protect microbial cells from denaturation and damage.

In addition to thermal lability, LAB have a very limited ability to synthesize osmoregulatory substances (osmolytes), and therefore have to obtain them from the external environment. Thanks to osmolytes, small, soluble organic molecules, the osmotic pressure is regulated. These substances also have a stabilizing effect on cell membranes and proteins, without reducing enzymatic activity, protecting the cell from increased temperature or drying out. Rapeseed meal is rich in osmoregulatory substances such as amino acids, in particular proline and short peptides (34).

The results are preliminary and will be continued.

Conclusions:

1. The low-temperature drying of fermented rapeseed meal stabilized the product and allowed it to be safely stored for 30 days without signs of mould.

2. The drying temperature of 48°C did not negatively affect the biological value of the nutrients.

3. The parameters selected for the drying process did not negatively affect the survival of the starter culture in dried rapeseed meal after its rehydration.

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