



## REVIEW

on a dissertation work for obtaining a scientific degree "Doctor of Science" in: field of higher education 5. Technical sciences, professional field 5.13. General engineering, scientific specialty "Technology of milk and dairy products"

**Author of the dissertation:** Assoc. prof. Tsend-Ayush Chuluunbat, PhD – Faculty of Food Technology and Biotechnology, Mongolian University of Science and Technology

**Topic of the dissertation:** "Theoretical and experimental justification of the technology of dairy products of functional nutrition in the conditions of Mongolia"

**Reviewer:** Prof. Gyurga Stefanova Mihaylova, DSc Trakia University - Stara Zagora, 5. Technical sciences, 5.12. Food technologies, scientific specialty: Technology of milk and dairy products,

Appointed as a member of the scientific jury by order No. RD-16-824/13.07.2022 of the Rector of AU - Plovdiv.

### 1. Brief introduction of the candidate.

Assoc. prof. Tsend-Ayush Chuluunbat, PhD was born in 1961. In 1985, she graduated higher education at the Moscow Technological Institute of Meat and Dairy Industry with a Master's educational and qualification degree, major "Technology of milk and dairy products". In 1997, she defended her thesis for the "Doctor's" educational and scientific degree in Technical Sciences at the East Siberian Technological University, Ulan-Ude, Russia. Both degrees ("Master's" and "Doctor") are recognized by the Academic Council of the AU - Plovdiv and are entered in the register of academic recognition of the NACID. During the period 1985-1989, she worked as a technologist at the Milk Production Factory in Ulaanbaatar, Mongolia. In 1989, she started working as a researcher at the Mongolian University of Science and Technology, later as a lecturer (1990-1991), senior lecturer (1992-2004) and associate professor – initially (2005-2014) at the Faculty of Food Technology and Biotechnology, and since 2014 to the present in the Faculty of Industrial Technology.

The competencies that Assoc. Prof. Tsend-Ayush indicates to own are: excellent knowledge of the Russian language and good command of English language, engineering skills in the field of milk and dairy products, computer skills, ability to work in a team, communication and good organizational skills. From 2002 to 2012, she conducted 7 specializations (on different programs and of different duration) in China, Belgium, Russia, Japan, Egypt and Korea. From the presented materials it can be seen that during her professional development she has gone through successive stages of preparation, during which she has acquired theoretical knowledge and practical skills in the fields of her scientific and teaching activities.

## **2. Relevance of the problem.**

The presented dissertation contains complex studies with a wide range of factors influencing the quality of the studied products which are of great importance for healthy nutrition. Dairy products are a group of basic foods preferred by different age groups, which is why they arouse interest not only among consumers, but also in the scientific research of specialists working in this direction. Taking into account the importance of providing humanity with dietary and medicinal dairy products, the dissertation acquires even greater importance, and the isolation of lactic acid bacteria with probiotic potential from traditional dairy products is a prerequisite for the development of preparations with prophylactic and therapeutic effect and a way to solve the objective set.

The scientific development is adapted to the conditions of Mongolia and is aimed at a specific object of research - milk, in view of the production of functional products by applying new starters, selected fermentation conditions and technological parameters, and evaluation of the obtained milk products. The theme is contemporary and relevant, and the obtained results are of interest not only to the science and practice in Mongolia, but also beyond its borders. My main arguments for this are as follows: the production of functional dairy products is becoming increasingly popular and in demand in the market; the use of goat's milk in Mongolia is expanding as a valuable dietary product against the background of the development of goat breeding in the country; there is insufficient research in the field of sheep's and goat's milk obtained from Mongolian breeds of animals as a raw material for industrial production of dairy products; there is a need to isolate and identify the strains of microorganisms from traditional Mongolian dairy products with proven probiotic potential and develop science-based technologies of functional dairy products. I think that the dissertation has scientific and theoretical value as it also provides information on the possibilities of creating dairy products containing protective factors, in particular probiotic microorganisms.

## **3. Aim, tasks, hypotheses and research methods.**

The purpose of the research is clearly formulated, corresponds to the topic of the dissertation and reflects the essence of the study. The 11 tasks set are sufficient for its implementation, well-grounded, logically connected and purposeful. The methodological basis for developing the dissertation is based on: a systematic approach, which includes defining the problem, setting the goal and tasks, developing a working hypothesis to achieve the goal, mathematical processing and analysis of the results, testing and implementation of new technological solutions.

The objects of the research and the main thesis of the author, which defines the need to develop technologies of functional dairy products based on the selection of bacteria with probiotic properties, are correctly defined. The thesis is realized through an in-depth study of the optimal conditions of milk fermentation by new starter cultures isolated from national Mongolian dairy products and search for technological solutions for the creation of new groups of probiotic, synbiotic and protein products with improved health characteristics.

From the described in chapter 3, one gets a real idea of the large-scale research work carried out. The research scheme and methodology are carefully presented. It is indicated that the experimental work has been carried out in scientific research laboratories in Mongolia, Japan, Moscow, South Korea and AU - Plovdiv, and in the production conditions of a number of enterprises in Mongolia and abroad. Since the topic is interdisciplinary, an extremely large set of classic and modern methods of assessment and analysis are used - physicochemical, biochemical, microbiological, organoleptic, genetic, statistical methods, technological part, etc. The procedures for isolation, identification and investigation of the properties of the strains are presented. The chosen methodology corresponds to the complex character of the study, correct experimental setups and appropriate methods of analysis, which are well described, are used. The huge volume of research carried out and the precision in its implementation make a huge impression. The presented is methodologically sound, the applied methods are consistent with the objectives of the research, and my assessment of this key, for every scientific work, section is high.

#### **4. Visualization and presentation of the obtained results.**

The dissertation is made up of 288 pages, and structurally, the requirements regarding the included sections are met – introduction, main part (including 9 chapters), conclusions, list of literature sources, appendices, contributions, as well as list of abbreviations.

The results are well illustrated with 77 tables and 28 figures, graphs, diagrams and photographic material. The data in the tables and figures are presented as averages or variances, with units of measure, with a legend where necessary, etc., making them easy to understand. The experimental results are documented in a more appropriate way and they are interpreted professionally, which is a proof of the author's qualities as a precise researcher. The scientific style is accurate, clear and understandable. The layout of the dissertation work, the scientific style and the correct reflection of the obtained results make a very good impression. The results and their discussion are presented in 5 sections, which to a large extent correspond to the tasks set for implementation.

#### **5. Discussion of results and references used.**

From the material presented in chapter 4 it is seen that the determination of the following has been carried out: chemical and amino acid composition of milk (goat, sheep and cow's) from Mongolian breeds of animals; fractional composition of whey proteins (goat and cow's milk); mineral composition (Ca, Na, K, Mg, P and Zn) of cow, goat and sheep's milk from pasture-raised animals; vitamin composition (vit. A, B and E) of milk (goat, sheep and cow's); safety (heavy metals, mycotoxins, antibiotics, radionuclides) of goat and sheep's milk from different regions of Mongolia.

The results of the isolation and research of strains of microorganisms are discussed in chapter 5, with 587 strains being identified from different dairy products (88 samples in total) prepared according to traditional technologies.

Studies in several directions regarding the qualities of lactic acid bacteria as probiotics have been presented. Assoc. Prof. Tsend-Ayush systematically describes the results related to the adhesive properties as one of the main criteria in the selection of probiotics. Another aspect of the scientific research is the tolerance of lactic acid bacteria to bile acids and gastric juice in connection with their use in the production of certain types of fermented milk products and the associated possibility of preventing certain diseases. The carbohydrate profile of strains of lactic acid bacteria selected as probiotics was determined. As a result of researching the microflora of dairy products selected from 4 regions of Mongolia, 10 homofermentative probiotic strains, which were used in the production of lactic acid products, were identified. The findings give reason to recommend the isolated and identified strains of lactic acid bacteria with probiotic properties for inclusion in the collection of microorganisms of Mongolia and the creation of starter cultures for the production of fermented milk products.

Proceeding from the fact that in Mongolia there is no organized production of starters, the author presents in chapter 6 data on the types of lactic acid microorganisms and the sources of their isolation as well as the influence of the type and dosage of the culture in the fermentation process of the starters. A comparative characterization of the starter cultures is made, their ratio in the combined cultures, the duration of fermentation, the accumulation of lactic acid and the changes in pH are determined. It has been shown that by the combination of different types of starters and regulation of the cultivation temperature, it is possible to obtain fermented milk products with desirable taste, texture and dietary properties. Through the evaluation of the probiotic properties of the selected strains from Mongolian dairy products, Assoc. Prof. Tsend-Ayush realized the task of creating new preparations with health benefits for consumers.

That is why the research carried out regarding the creation of technologies for probiotic, protein and synbiotic products, based on selected combined starter cultures and such from pure cultures, described in detail in chapter 7, are examples of the practical application of the results achieved in the selection of strains with probiotic properties. Technologies for lactic acid products based on goat's milk and technology for soft cheese have been developed, and their physicochemical characteristics have been made. A technology for brine cheese from sheep's milk, a technology for cow's yogurt with probiotic properties, as well as a technological scheme for the production of cottage cheese are presented. The antioxidant activity and polyphenol content of yogurt with the addition of green olive powder is evaluated. A technology for synbiotic dairy products has been developed and a technological scheme for the production of lactic acid products with prebiotics, in this case inulin and fructooligosaccharides, has been presented, as a result of which it is concluded that such prebiotics are suitable for use in the production of fermented dairy products for the improvement of the organoleptic, microbiological, functional and structural-mechanical indicators. The technological research carried out shows the effectiveness and prospects of using the strains of lactic acid bacteria isolated from traditional Mongolian dairy products to create functional products with probiotic properties.

In the last stage of the work, chapter 8, the results of a clinical trial of the probiotic strains are presented. The influence of lactic acid bacteria isolated from Mongolian dairy products on the growth of *Helicobacter pylori* in *in vitro* experiments is investigated, and *in vivo* data on their effect on mice infected with *Helicobacter pylori* are also presented. The clinical studies end with the evaluation of the properties of the probiotics isolated from Mongolian dairy products on defecation in women aged 18 to 39 years.

On the basis of the studies and analyzes carried out 9 conclusions are made, which derive from the results obtained and have scientific and practical significance and a perspective for direct application in practice.

The literature used covers 257 titles, 195 of which are in Latin. The bibliographic reference includes 19 normative documents and 10 publications by Assoc. Prof. Tsend-Ayush, and 46% of the cited sources are from the last 10 years. The literature review is a description of the achievements of a number of authors who have worked on various aspects of: the medico-biological rationale for the creation of functional nutrition products; the state of goat and sheep farming; scientific and technological aspects for the development of functional dairy products; characterization of national Mongolian dairy products and microflora of probiotic products. The analysis of the literature allows the identification of the objective prerequisites for conducting extended scientific and applied research for the creation of functional dairy products technologies, which justifies the development of the present work.

## **6. Contributions of the dissertation.**

Generally evaluated, the dissertation work of Assoc. Prof. Tsend-Ayush, PhD has the character of an original scientific and applied work, which improves the knowledge about the influence of added probiotic bacteria on the biological characteristics of the dairy products and substantiates the principles of selection of strains of microorganisms for starter cultures for functional dairy products. A significant part of the results is related to obtaining new knowledge in the study of the probiotic properties of lactic acid bacteria. The author has created a reference with 6 contributions defined as original and possessing theoretical and scientific-applied significance, which I accept by systematizing them into 2 groups:

### **Scientific contributions**

The following can be cited as original scientific contributions:

For the first time, a complex study has been carried out on the properties of microorganisms isolated from Mongolian fermented milk products prepared according to traditional technologies.

The composition of the microorganisms isolated from national Mongolian fermented milk products for starters production is scientifically substantiated.

Bacterial strains with proven probiotic potential are selected in view of their use as starters for the production of dairy products with improved health characteristics.

Strains of lactic acid bacteria with high probiotic activity are isolated and identified and classified as *Lactobacillus plantarum* and *L. paracasei* spp. *paracasei*.

Clinical trials with fermented milk products have been conducted with referenced to antihelicobacterial and therapeutic activity in the gastrointestinal tract.

### **Scientific and applied contributions**

The scientific-applied contributions that are contained in the results are: Starter cultures for fermented milk products with health benefits and the technological parameters for their production are described.

A technology has been developed for the preparation of starters containing cultures of lactic acid bacteria with probiotic properties isolated from Mongolian national fermented milk products.

Optimal conditions for milk fermentation with new starters have been selected, and the main technological parameters for the production of fermented products from goat, sheep and cow's milk have been substantiated.

New groups of probiotic, synbiotic and protein products characterized by increased nutritional and biological value have been developed.

It was found that *L. paracasei spp. paracasei* (06TSD19b) isolated from Mongolian fermented milk products not only has a probiotic effect but it also shows anti-helicobacter activity.

The scientific contributions are related to the enrichment of science with new knowledge, and the scientific-applied ones are related to the selection of probiotic strains and the development of a number of fermented milk products with probiotic properties, implemented in Mongolia and abroad. The results, which are supported by author certificates and patents (7 pcs.), have an important applied significance as new technologies have been proposed.

### **7. Critical notes and questions.**

I have no significant notes and recommendations for the presented dissertation, since most of the proposals for changes made in the preliminary examination have been taken into account by Assoc. Prof. Tsend-Ayush. I have a note of a technical nature regarding the auto-referral, which lists 12 tasks instead of 11 (there is a repetition of task 9 and 10).

### **8. Published articles and citations.**

36 scientific publications are presented in connection with the dissertation, 10 of which are referenced in journals and indexed in world-famous databases with scientific information (Scopus and Web of Science). The remaining 26 publications are in peer-reviewed journals or in edited collective volumes, of which 12 pcs. are in foreign editions. The publications reflect the independence of the author - 11 pcs. are individual ones, and in other 11 pcs. – she is the first author. 5 patents and 2 copyright certificates are attached. A list of participation in 8 projects (international and national), as well as 3 monographs (one individual and two collective) is presented. Assoc. Prof. Tsend-Ayush presents 92 citations in refereed and indexed journals and 20 citations in non-refereed journals with scientific review.

The abstract objectively reflects the content of the dissertation work; it is made up of 60 pages and corresponds to the structure of the dissertation.

### **CONCLUSION:**

The research has been conducted, discussed and summarized at a high scientific level in accordance with the current achievements on the role and use of lactic acid bacteria in gastrointestinal infections and disorders, and represents an original contribution to the science of probiotics and functional dairy foods. As a result of the conducted research, fermented milk products with health benefits based on selected probiotic bacteria have been developed and put into practice.

The report on the fulfillment of the minimum national requirements for the award of the Science degree "Doctor of Science" in field 5. Technical Sciences shows that the candidate covers the required 50 points from group A related to the educational and scientific degree "Doctor". Under the indicators from group D, 36 publications and 3 monographs are indicated, with a total amount of 356.4 points out of the required 100 points, and in group E the citations form 960 points out of the required 100 points.

Based on the analysis made, the originality of the results obtained and the contributions, I think that the presented dissertation meets the requirements of the LDASRB and the Rules of the Agricultural University for its application, which gives me reason to evaluate it positively.

I allow myself to propose to the honorable Scientific Jury to also vote positively and to award to Assoc. Prof. Tsend-Ayush Chuluunbat, PhD the scientific degree "**Doctor of Science**" in the field of higher education 5. Technical sciences, professional field 5.13 General engineering, the scientific specialty "Technology of milk and dairy products".

**Date:** 07.09.2022  
Stara Zagora

**REVIEWER:** .....  
(Prof. Gyurga Mihaylova, DSc)