



OPINION

Regarding a dissertation thesis for acquiring the scientific degree 'Doctor of Science' in the higher education area 5. Technical sciences, professional field 5.13 General Engineering, scientific specialty 'Milk and Dairy Products Technology'.

Author of the dissertation thesis: Chuluunbat Tsend- Ayuush, PhD in an independent form of preparation at the department of 'Animal Science' at Agricultural University Plovdiv.

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

Thesis Advisors: 1. Prof. D-r Iliana Nacheva
2 Prof. D-r Vasil Nikolov

Reviewer: Prof. Todor Dimitrov Dimitrov, DSc Faculty of Agricultural Sciences at Trakia University Stara Zagora under 5. Technical Sciences, 5.12 Food Technologies, Milk and Dairy Products Technology, chosen as a member of the scientific jury following regulation 16-824/ 13.07.2022 of the Rector of Agricultural University Plovdiv.

The dissertation thesis submitted contains eight chapters including: introduction, analytical overview of the literature used, methodical part, results and discussion, conclusion and contributions.

1. Relevance of the Topic

Healthy food production issues have been of extreme importance for the mankind in the recent years. More and more information about the use of different additives with the purpose of improving the quality and the functionality of the food can be found in the literature.

The appropriate literature awareness of the doctoral student (she has used 257 literature sources) correctly directs her towards the unsolved problems regarding the functional dairy products production in Mongolia.

The problems which have not been solved yet are chiefly limited to unsound scientific technologies used for the production of probiotic and synbiotic baby dairy products and starter cultures. In addition, as it is indicated in the dissertation thesis, the industrial production of goat's and sheep's milk products is not nearly as well developed and examined as that of the cow's milk products. There are no scientifically justified technologies elaborated for the former's production. Therefore, the issue concerning the development of scientifically based technologies for the production of probiotic and synbiotic dairy products from goat's and sheep's milk containing beneficial microflora for colonization of the gastro-intestinal tract is topical and has an important medical significance (for the purpose of inhibiting *Helicobacter pylori* and enterobacteria).

The attempts for isolation and identification of probiotic strains from the traditional Mongolian dairy products have an equal importance in the present dissertation thesis.

2. Aim, Tasks, Hypotheses and Methods of Examination

The aim of the dissertation thesis is well- founded and it naturally stems from the relevance of the problems which need to be solved and which are limited to the development, scientific substantiation and creation of functional dairy products with the use of new types of starter cultures prepared from lactic acid bacteria which are isolated from Mongolian national dairy products.

The twelve tasks reasonably derive from the well-formulated aim of the dissertation thesis.

The subject of the study are aggregate samples of goat, sheep and cow's milk received from privately-owned farms. In addition, some Mongolian national fermented milk products: tarag (identical with yoghurt); hoormog (a fermented camel's milk product); airag (a fermented mare's milk drink); byaslag (a type of cheese), etc, were also examined. Strains from national Mongolian dairy products were used for the creation of starter cultures.

During the study, standards and modern microbiological and genetic methods have been used. Furthermore, conventional physicochemical, biochemical and rheological methods as well as mathematical methods, statistical processing of the results, and images with mathematical models have also been applied.

3. Visualisation and Presentation of the Results Obtained

The results obtained are visualized in 73 tables, 25 graphs and diagrams which correctly and precisely report the data obtained. What is more, they are skilfully interpreted and commented.

4. Discussion of the Results and the Literature Used

4.1. The physicochemical content and the safety of the milk of some Mongolian farm-animal breeds was studied. It was ascertained that the milk of the local Mongolian cattle is characterized by high levels of dry matter, fatty substances and protein percentage. It was found that the share of the essential amino acids in the total protein of the goat's, sheep's and cow's milk of the Mongolian cattle breeds slightly differ- respectively 43,64%, 45, 41% and 44,19%. The results show that the goat's milk has a higher content of β - globulin and α -lactalbumin than the cow's milk, especially of α -lactalbumin. With reference to the minerals, the goat's milk is richer in calcium than the cow's milk but it is secondary to the sheep's milk. The goat's milk has a slightly higher content of important micro and macro elements such as Na, Ca, Mg and P with K being the only exception. The sheep's milk has a considerably higher content of vitamins B₁ and B₂ when compared to the goat's milk and the cow's milk. Similar results are reported for vitamins A and E.

4.2 Isolation and identification of lactic acid bacteria strains and studying the probiotic properties

One of the many good aspects of the dissertation thesis are the attempts for identification of the microflora of the national dairy products produced through traditional technologies. Apart from the various types of lactobacillus and coccus (the exact number is specified in the dissertation thesis), in 88 fermented milk products samples (tarag, hoormog, airag, aaruul, byaslag, etc.) yeast was also found.

The results regarding the ascertainment of the composition of the lactic acid bacteria and the probiotic activity of the strains check are an extremely important element of the study and lay the foundation for the production of Mongolian lactic acid probiotic starter cultures necessary for the Mongolian dairy industry.

The microorganism selection, their differentiation, the probiotic activity testing and the starter culture preparation testing were all performed in the appropriate sequencing following the latest methods, compatibility and combination.

The Mongolian starter cultures production gives the opportunity new functional fermented milk products, which are both relevant for Mongolia and in compliance with the world trends, to be created.

The microorganism selection used during the tests was scientifically based. The optimal temperatures (38,42 and 45 °C), the quantity of the starters (3, 5 and 8%) as well as the end pH- 4,6 before cooling down were also ascertained. Thus, their optimal dose 3-8% and the acidification period- up to 4,5 h are specified. Upon use of starter cultures containing STr.

Salivarius subsp. Thermophiles strain TSI/1216, Lactobacillus helveticus TSDI/11 and Lactobacillus fermentum DTS/143, it was ascertained that Lactobacillus helveticus TSDI/11 is the most active.

One of the most important criteria for the starter cultures testing is the number of the microorganisms upon coagulum formation i.e. the quantity of the lactic acid separated and the pH change in their stationary phase.

The results of the 10 lactic acid bacteria strains studied indicated different active acidity, milk acid produced and different log 10 KOE/ml.

The data show that at the end of the fermentation process of all starter cultures used, their viable cell count was from 7,1 to 8,9 log 10 KOE/ml. The *L. paracasei* subsp. *paracasei* 06TSD19 strain had a high cell count ($8,9 \pm 0,1$; $7,8 \pm 0,24$) and milk acid quantity $0,533 \pm 0,08\%$, and the lowest producer was *L. plantarum* 05DTS23, *L. plantarum* 06LH₂.

4.3. According to the doctoral student, the results obtained indicate the appropriateness of the starter cultures prepared from the *L. paracasei* subsp. *paracasei* 06TSD19 and *L. delbrueckii* subsp. *lactis* 06DTS3 to be used for fermented products, namely yoghurt.

As the doctoral student has rightly stated, the elaboration of new concepts with reference to the probiotics and the functional nutrition affects many fundamental and nutrition aspects related to the healthy eating, medicine, biotechnologies, etc. In this respect, the problems mentioned above may be considered relevant for Mongolia.

Experiments were carried out aimed at the joint cultivation of bifidobacteria and lactobacilli acidophilus for the purpose of creating a probiotic product (table 14). The data show a ratio of 2:1 from a technological point of view as the technological process is shortened to 4,5h.

A high biological sufficiency is ascertained upon the production of fermented goat's milk dairy products with three types of starter cultures.

The multiple experiments conducted by the doctoral student and the good results allow the scientifically-based specification of the technological parameters of the fermented goat's milk products on the basis of probiotic lactic acid bacteria isolated from Mongolian national dairy products.

The experiments performed resulted in the creation of new probiotic starter cultures called 'Shim' and the production of soft goat's milk cheese made from them. An enhanced technology based on an existing one was elaborated with reference to the production of 'Branza' sheep's milk cheese with 41% fatty substances in the dry matter and an input of 1 kg per 4,5 l of milk. The *L. paracasei* strains (from the national dairy products) and functional lactic acid foods (whey proteins, glucose and gelatine) were used upon the elaboration of the new technologies for production of yoghurt with probiotic properties. A technology for yoghurt production with the addition of green olives powder was also developed. An analysis of the polyphenolic compounds content and the antioxidant activity of the product was performed. It was ascertained that the polyphenolic compounds decrease in the course of storage period.

The development of technologies for synbiotic fermented milk products for prevention of diseases spread in the monitored ecological regions is, in my opinion, one of the most considerable merits of the dissertation thesis.

Inulin and fructooligosaccharides are used in view of the fact that they are the most important prebiotics. It was ascertained that the addition of up to 2% of inulin and starter cultures in combination with lactic acid streptococci, lactobacilli and bifidobacteria in a 2:1:1 proportion is appropriate.

The studies on the *Helicobacter pylori* growth suppression through lactic acid bacteria strains in the gastrointestinal tract also deserve admiration. It was ascertained that the

Helicobacter pylory growth suppression depends not on the type of microorganisms but precisely on the starter culture strain used.

The author has created new technologies for probiotic and synbiotic cultures isolation and has obtained the respective certificates.

10 scientific publications in journals, which are referenced and indexed in world-famous database of scientific information, have been submitted throughout the elaboration of the dissertation. All of the publications were submitted after 2011 and can be found in renowned food industry journals.

The scientific publications with a scientific- peer review published in non-referenced magazines are 26, there are three monographs, seven patents and copyright certificates.

All materials and literature sources published in the dissertation thesis have given the doctoral student the opportunity to submit a comprehensive scientific work which is rich in information and can be seen as an example for development of researches related to the food industry.

5. Dissertation Thesis Contributions

There are a total of six contributions- two of which are original, with a theoretical and scientific significance with reference to the nutrition as they are related to the isolation of lactic acid bacteria strains with high probiotic activity, and the other four have a scientific importance.

The nine conclusions reported in the dissertation thesis derive naturally from the results obtained.

I have no objections regarding the conclusions and contributions submitted.

6. I take the liberty to express my immense satisfaction from the research submitted and I have no critical comments.

7. Publications, articles and citations

39 scientific papers and 90 citations have been presented in relation to the dissertation thesis.

The autoreferate provided objectively reflects the structure of the dissertation thesis.

8. Conclusion

Based on the scientific and other different methods of research applied by the doctoral student, the correctly performed experiments, the overviews and conclusions made, the contributions for the science and the practice, I consider that the dissertation thesis submitted complies with the Development of the Academic Staff in the Republic of Bulgaria Act and the Agricultural University Regulation on its implementation, which leads me to convincingly evaluate it positively.

I allow myself to suggest the honourable scientific jury to also vote positively and award Chuluunbat Tsend- Ayuush the scientific degree 'Doctor of Science' in the higher education area 5. 'Technical sciences', professional field 5.13 'General Engineering', scientific specialty 'Milk and Dairy Products Technology'.

26.08.2022
Stara Zagora

Reviewer: 
Prof. Todor Dimitrov, DSc