

MANUFACTURE OF TRADITIONAL B&H CHEESES WITH SELECTED INDIGENOUS BACTERIAL CULTURES AND TECHNOLOGICAL PARAMETERS AS BASIS FOR INDUSTRIAL PRODUCTION

Previous project: Standardization of Technology and Chemical,
Physical and Microbiological Quality Characteristics of Autochthonous
White Pickled (Travnik) and Hard (Livno) Cheese in Bosnia &
Herzegovina

Project co-ordinators:

UMB: Roger Abrahamsen -> Judith Narvhus
Univ. of Sarajevo: Zlatan Saric



University of Sarajevo : IKBM, UMB

1. Background

- Faculty of Agriculture and Food Sciences (FAFS) the oldest one in B&H (1940)
- Academy Prof. Nikola Zdanovski established Dairy Science Chair
- Research topics through years are traditional dairy products
- 1992. FAFS burned down and completely devastated
- 1996. 1st moving - moved to Campus building
- 2008. 2nd moving – moved to another Campus building



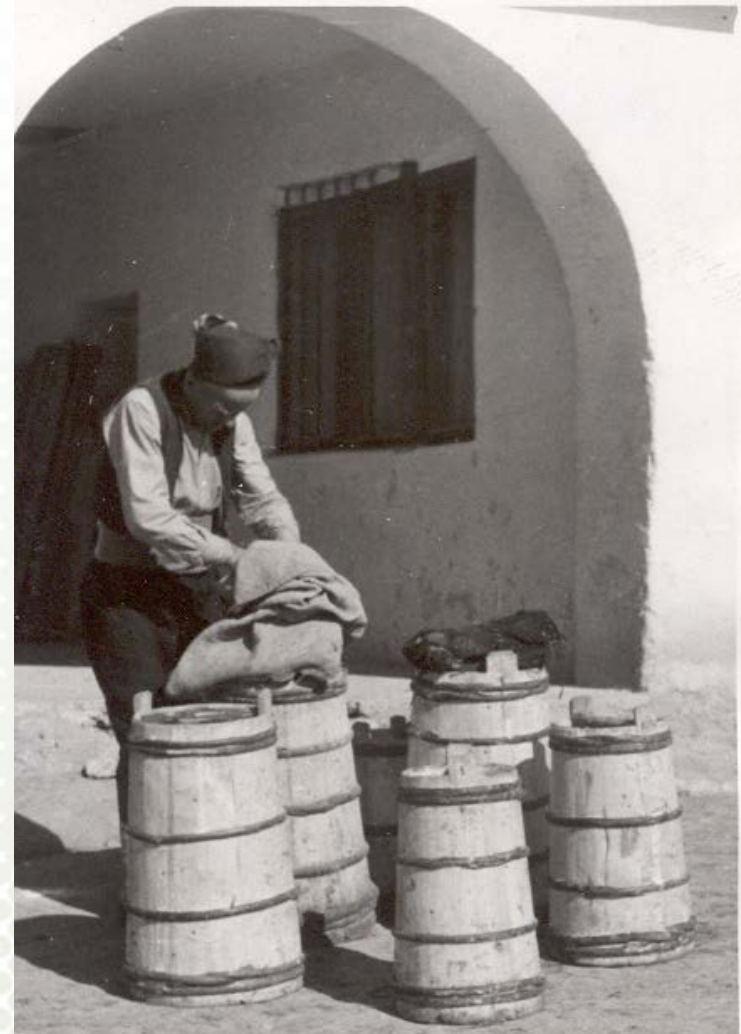
1. Background

- After the war – help from ETH, Switzerland (prof. Puhar program) and Dutch Government
- 2006. cooperation UMB and FAFS
Project 3. Standardization of Technology and Chemical, Physical and Microbiological Quality Characteristics of Autochthonous White Pickled (*Travnički*) and Hard (*Livanjski*) Cheese in Bosnia & Herzegovina (Norwegian Ministry of Foreign Affairs)
- During this project main characteristics of chosen cheeses are determined and main courses of further research



1. Background

- Most important B&H cheeses are **Livno** and **Travnik** cheese that are produced from raw milk, no selected starter culture
- Significant variations in quality characteristics and generally poor hygienic level
- These cheeses are characterized by a complex microflora, which is responsible for creation of cheese aroma during the ripening.
- Problems related to production and sale of raw milk cheeses in B&H has led to a significant reduction in the number of producers, and almost disappearance of this type of production.





TRAVNIK CHEESE



LIVNO CHEESE

- During first project with help of UMB scientists new research topics were revealed (importance of salt for proteolysis, organic acids, volatile components)
- Young scientists from FAFS were educated by UMB people in liquid/gas chromatography (HPLC/GC) and statistics methods (Unscrambler)
- Exchange of students was realised (one norwegian Master student defended Msc work at BiH cheese (Audun Gunnes) and also 4 BiH students)
- Main conclusion was UNSTANDARDIZED cheese, it was decided to start isolation of natural microbial population in order to use it in industrial production



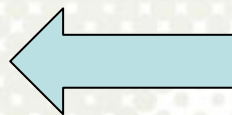
Following examples of other countries.....



Italy



France

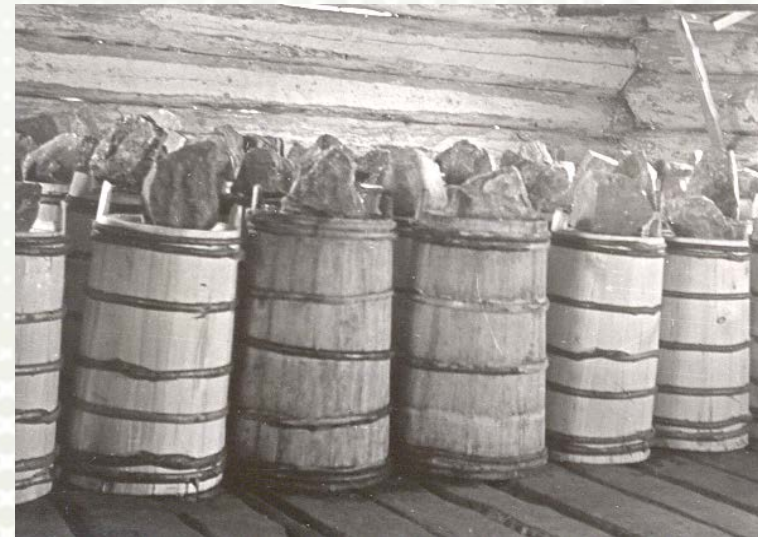


Switzerland



Main goals

- Isolation, identification and characterization of natural micro-flora in the chosen traditional cheeses
- Strengthening the scientific capacity through cooperation of Bosnian and Norwegian Scientific/Educational Institutions
- Improving the technical capacities of Laboratory of Dairy Science at Faculty of Agriculture and Food Sciences (FAFS), Sarajevo
- Formation of culture collections B&H traditional dairy products and the preservation of these organisms
- Realization of the assumptions for the application of traditional cheeses isolates in industrial conditions www.umb.no



Objectives

- Isolation, identification and characterization of natural microflora in the chosen traditional cheeses (*Livno* and *Travnik* cheese)
- Combination of selected isolated LAB in order to find appropriate starter culture which would give optimal cheese quality with elements of source technology
- Improvement and standardization of technology parameters applying experimental cheese manufacture in order to get uniform high cheese quality
- Investigation on traditional dairy products in order to save biodiversity in the world and facilitate rural development

Livno Cheese

- 2010
 - Five producers of high quality cheese selected
 - Cheese obtained after salting, sampled after 1, 30 60 and 90 days
 - Microbiological and sensory analysis
 - Chemical and physical analysis
 - Ca. 1200 isolates of microorganisms
- 2011
 - Best three producers selected for second sampling
 - Similar sampling as in 2010

Livno Cheese: Analyses on isolates

- Catalase test,
 - Gram staining
 - the formation of gas from glucose
 - Growth at 15 ° C and 45 ° C, and in 2 and 6.5% salt
 - Milk acidification after 8 and 24h at 30 ° C.
 - Sensory properties after 24h growth in milk, smell and quality of cheese curd.
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- October – December 2011 UMB Tarik Dizdarevic
 - Identification by molecular techniques
 - Technological properties

- cooperation between UMB and FAFS October 2011/2012-B&H partner was trained in molecular biology
- **30 Livno cheese** (0, 1, 30, 60 and 90 days) from five cheese manufacturer was sampled. pH average of 5.35. The average sensory score was 15.41.
- Hygienic quality of **Livno cheese** was tested. Bad hygienic indicators are decreased during ripening
- Isolation of LAB from **Livno cheese** included: *Lactococcus* spp., *Lactobacillus* spp., *Enterococcus* spp., *Leuconostoc* spp. 1,200 isolates obtained from **Livno cheese** were purified and frozen at -83°C..
- Purification, Gram staining and physiological tests were performed on isolates, and the best strains were selected for further analysis
Physiological tests showed that microorganisms from traditional products are more resistant to higher temperatures and the higher concentrations of of salt.
- Due to the poor quality it Livno cheese from two manufacturers, it was decided in 2011 to repeat the experiment with the top three producers in 2010 using the same experimental design

- Selection of isolates from 2010/2011 had been performed on the basis of physiological tests and 370 selected isolates were shipped to Norway.
- Dominant LAB groups during ripening were found by independent methods (PCR-DGGE). It is found differences in the dominant microbial population belonged to the group Lactococci, *Lb. casei*, *Lb. plantarum*, *Pediococcus* etc
- Further selection had been done with HRM-PCR, based on the melting point of DNA isolates. HRM-PCR showed statistically differences between isolates, and 85 isolates were selected for sequencing.
- Selected isolates (85) were sequenced. Results identified a large number of microorganisms that form a complex microflora of Livno cheese

- In order to determine the biochemical characteristics, 14 isolates out of the total, were selected for experiment with cheese slurries. Slurries were subsequently monitored during incubation at 30°C within 7 days (equivalent to cheese ripening period of 2 months)
- The cheese slurry samples were analyzed amount of sugar, organic acids, amino acids and volatile compounds were measured. After 7 days of incubation biggest number of bacteria was determined in cheese slurry with inocula *Lb. Case* / *Lb.paracasei*, *Pediococcus* and *Lc. lactis* subsp. *Cremoris*
- Domination of total organic acids was observed in samples from a group of *Lc. lactis*, *Lb. plantarum*, *Pediococcus* while total amino acids were characteristic of *Lb. plantarum*, *Leuconostoc* and *Streptococcus macedonicus*.

Travnik cheese

- Studied in the previous project:
- Dominant lactic acid bacteria isolated – *Enterococcus* & *Leuconostoc*
- 2011 (totally three producing years)
- Three producers selected
- Further isolations made during maturation
- Indicators of hygiene
- Cheese stored for analysis
- Isolates to be identified

- **Travnik cheese** aged 0, 1, 30, 60 and 90 days from three selected high-quality cheese manufacturer was sampled within two consecutive years (2011/2012) - 900 isolates obtained from **Travnik cheese** were purified twice and frozen at -83°C and identical tests were made (purification, Gram staining and physiological tests) as well as for **Livno cheese**.
- In order to optimize the amount of salt in **Travnik cheese**, samples of whey/brine from different wooden tubes of three high quality cheese manufacturers were taken and analyzed (pH, acidity, % NaCl, a_w , % DM).
- Experiment was set at one of **Travnik cheese** producers where the cheese was salted with different concentrations of NaCl, 2.5%, 4.0% and 5.5%; chemical analysis are made after 10, 20 and 30 days of ripening period. In order to check the penetration of salt in the cheese slices the salt content is measured in the middle and at the end of cheese slices. All mature cheeses will be sensory evaluated. - There was a effect of salt only on the total number of Staphylococcus spp.
- Amount of salt has a impact on the external appearance of the cheese, consistency, smell and taste. Ripening period has a statistically significant impact on the appearance and smell of cheese.

- **Currently two Ph.D. are doing part of their research at UMB:**
- 1. Final cheesemaking experiments with selected starter microorganisms
- 2. Identification and characterization of bacterial population isolated from **Travnik cheese** by molecular methods

- **Further activities at chosen identified starter microorganisms (Master students):**
- Antibiotic tests
- Microbial population growth in selected combinations
- Resistance at bile salts and low pH (probiotic properties)
- Amount of salt has an impact on the external appearance of the cheese, consistency, smell and taste. Ripening period has a statistically significant impact on the appearance and smell of cheese.

Practical use and cooperation

- Practical use of selected starter culture in industrial production of selected cheeses with possibility to use pasteurization and at the same time to save traditional microflora and therefore cheese characteristics
- Current cooperation with Livno and Travnik cheese association
- Future cooperation with small scale cheese industry (two dairies in Livno)

Improving the technical capacities at Laboratory of Dairy Science at Faculty of Agriculture and Food Sciences (FAFS), Sarajevo

- *Kjeldahl system*
- *Digestion chamber*
- *Ultrafrizer (-83°C)*

The equipment were installed and currently serves to educate students and for research purpose.



Scientific activities

Publications:

- *„Determination of Sensory Characteristics and Volatile Components Content in Traditional Travnik Cheese“*
- T. Dizdarević, Z. Sarić, R.K. Abrahamsen , J. Narvhus²
- IDF Cheese Ripening Symposium, USA, Wisconsin, 2012.
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- **“Technology and quality characteristics of traditional Livno cheese”**
- Z.Sarić, T.Dizdarevic, A. Tole, A. Safić, L. Spiljak, J.Narhvus, R.K.Abrahamsen. Experts Congress on Agriculture and Food Industry, Izmir, Turkey, 2012.
- **“An Atlas of Sheep Cheese of the Countries of Western Balkans”**
- Havranek J., Mikulec N., Antunac N., Iveković D., Jakša I., Rogelj I., Perko B., Trmić A., Čanžek-Majhenić A., Sarić Z., Dizdarević T., Popović-Vranješ A. Cvetanović D.
- University press Zagreb, Croatia, 2012

Educational activities in the project and building of human capacities

- Besides scientific and economical means, HERD is important for engaging of students (M.Sc. And Ph.D.)
- Therefore, students either have to pay themselves for doing thesis or they can be included in some projects
- Before 1992. budget for science amounted for 1,5% GDP, while today it is 30 times less i.e. 0.05%, as result FBH funds for scientific projects are low even if you get it at all

Educational activities in the project and building of human capacities

- Engaging students and making their theses on project topics
- Through the research activities young apprentice B&H partner was trained in molecular biology. The project included two Ph.D. students and 8 Master theses were or will be realized in the near future. This means great support for building of young scientific man power at FAFS, Sarajevo. Two Ph.D. Students are currently at UMB doing research within thesis
- This project has achieved synergy with the project SEE-ERA.NET Plus (Code SEEERAPLUS-133), entitled "Characterisation and tracking the origin of specific features of traditional cheeses in the Western Balkans," funded by the European Union.

THANK YOU!!!

