

Milk & Dairy products

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Portorož, Slovenia



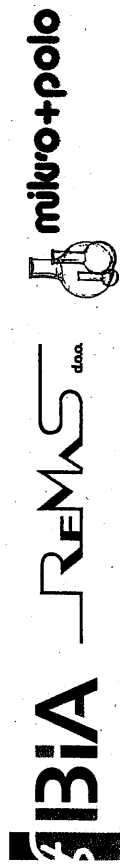
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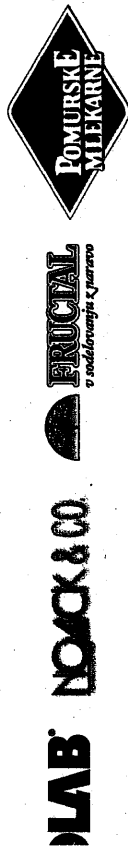
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Contents

a:	New Approaches in Milk Production and Processing	9
	Plenary lectures	11
	Invited lectures	13
	Short lectures	19
	Posters	25
b:	Food Safety - Risks and Quality Assurance - New Analytical Tools	33
	Plenary lecture	35
	Invited lectures	36
	Short lectures	45
	Posters	54
c:	Origin and Authenticity of Dairy Products	67
	Plenary lecture	69
	Invited lectures	70
	Short lectures	75
	Posters	85
d:	CAP Reform, WTO and EU Enlargement - Challenges for the Dairy Industry	95
	Invited lectures	97
	Poster	101
	Author index	102
	Advertisements	104

Milk production in the post genomic age

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Quantitative traits describing ability of dairy animals to produce large amounts of high quality milk were subject of intense selection work in the past. The majority of breeding programmes was focused on milk yield, and fat and protein content. The composition of protein fraction and its impact on manufacturing properties were frequently overlooked. In the traditional animal breeding phenotypic variation in these complex traits was explained using infinitesimal model. Identification of lactoprotein loci at molecular level, discovery of allelic effects for a number of lactoprotein genes and advances in physiology, endocrinology, cell biology and molecular genetics established a knowledge base which made the search for causal genes, involved in the regulation of complex milk traits a possible endeavour. In addition to lactoprotein genes, there are a number of genes contributing to milk traits. The introduction of QTL concept enabled identification and mapping of genomic regions with detectable effects on milk traits. Within these regions some milk trait candidate genes were identified using molecular cloning and comparative mapping approach. Recently, powerful genomic and proteomic tools have been developed which enable study of differentially expressed genes in different lactation stages and at different level of production. Micro array technology and 2D protein electrophoresis coupled with mass spectroscopy have potential to identify a large number of genes which are up- or down regulated during lactation. This represents a real possibility to verify phenotypic effects of candidate genes identified by QTL approach. Analysis of expression profiles can reveal important interactions among genes and elucidate complex regulatory pathways which are responsible for economically important milk traits. An overview, connecting different approaches and analytical tools with potential to identify complex genetic background of milk production, will be presented.

a

New Approaches in
Milk Production and
Processing

Non thermal milk processing technologies

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Commercial thermal processing can destroy milk qualities such as nutrients, flavour, and texture. Although different principles of action apply, nonthermal technologies such as high hydrostatic pressure (HHP), high intensity electric field pulses (PEF) that have detrimental effects on biological structures of microorganisms also shows some changes on food matrices. Structural changes can also be induced due to enhanced residual enzyme activities during and after treatment. In some cases the possibility to obtain desired novel effect on a food matrix can generate a real interest regarding the application of technology. HHP is an example of this situation. The application of the process leads to products where some quality attributes are enhanced over the others. The global advantages of this process in terms of quality are still not completely evaluated.

Although HHP can be used to decrease the microbial load in milk to eliminate pathogenic bacteria and some viruses, the effect of the pressure during this pressurization period may be seen on hydrophobic bonds of milk proteins as alteration of the subcellular structure of casein micelles. The changes on the conformational structure of the proteins indicate increased exposure of hydrophobic groups, which may also alter the functional properties of the system. Hence, the foaming, emulsifying and water binding capacities of the casein proteins may be influenced.

Studies has shown that, though at lower pressures (40-60 MPa) a delay in milk curdling but increased curd rigidity is predominant, at higher pressures (500-700MPa) rearrangement and compression of casein micelles that results smaller particles with greater hydrophobic character favour rennet coagulation.

Shelf-life studies of HHP processed milk has also indicated that, fat and protein content of milk had an influence on inactivation of bacteria. Higher protein and fat content has increased the resistance of target pathogens studied. Studies have also indicated two different type of injury that may mislead the processors on the safety of the HHP processed milk.

Milk composition and milk quality: the impact of nutrition

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Gross milk composition affects milk nutritive value and cheese making characteristics. The impact of animal nutrition on milk composition as well as manufacturing properties of milk will be presented. Despite the «major» protein, lipid and lactose components, in the past decade more and more attention have been addressed to a huge number of «minor» components that are of crucial importance for milk quality. A number of bioactive molecules, present in the milk protein fraction (lactoferrin, peptides and growth factors) have been identified. The most interesting components in the lipid fraction are vitamins and conjugated linoleic acid - CLA, due to its potential anti-carcinogenic, anti-atherogenic, anti-diabetogenic and immuno stimulatory effects. Components of the protein and lipid fraction, together with some milk enzymes, represent a network of molecules that provide milk and dairy products its unique and «extra nutritional» characteristics. Some of these components will be discussed in more detail, in particular their role in the nutrition of new born as well as adult consumer.

Organic dairying: a new challenge - or back to Stone Age processing?

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Production systems in agriculture are changing rapidly with progress in national and international efforts towards a sustainable agriculture. The benefits and limits of organic agriculture were documented in investigations covering more than 20 years of comparable production (conventional agricultural practice and organic agriculture) (Mäder P. et al. 2002). Within that context and together with consumer's concern about healthy food and environmental impacts the organic food market is growing at double digit numbers in several European countries as well as in North America. But the market share of organic products is different from country to country. Often dairy products are among the most important organic foods.

Several branch specific organisations established programs which allow a strategic positioning of their food range in the sense of a "food-health-wellness" strategy. Concern for healthiness is obviously still the most important reason for purchasing organic food, followed by taste (Ex-Preysch B et al. 2000). There is no question that processing has an impact on such properties. Codex Alimentarius and national food legislation cover primarily production of organic food but not the processing aspects, except for some restrictions with respect to the application of additives. For the aspect of environmental effects of food production life cycle assessment (LCA) has proved to be a useful method to track down main differences in potential environmental impact (Jungbluth et al. 2000). The most important aspect of organic food processing can be summarised under the title of minimal processing. International bodies as well as private organisations try to elaborate concepts for minimal processing, careful treatment and definition of authenticity. Any success in this regard will depend largely on agreements with certain standards. The preservation of genuine properties in food processing is a challenge for the food industry and research. Natural properties include bio-activity of the genuine food components. Ongoing research on preservation of bio active compounds follows the need of organic food products but, at the same time, covers important questions for the whole food industry.

Claiming better physiologically functional properties for organic foods could be an adversarial approach (good food/ bad food) hindering developments regarding traditional or novel foods. Another challenge for all partners in the food and nutrition sector is to minimise such negative effects.

Beyond processing, organic food creates new requirements on the analytical side. Research has to develop criteria to maximise the effectiveness of both production and processing at each transformation level, as well as methods to trace and guarantee the history, safety and authenticity of the product.

References

- Mäder P., Fliessbach A., Dubois D., Gunst L., Fried P., Niggli U. (2002) Soil Fertility and Biodiversity in Organic Farming. Science 298, 1694-1697
- Ex-Preysch B., Mühlemann-Pescalle, Mühler-Becker D. (2000) Nutri-Trend-Study 2000 Nestlé Suisse S.A. With Swiss Federal Public Health Office
- Jungbluth N. (2000) "Environmental Consequences of Food Consumption: A Modular Life Cycle Assessment to Evaluate Product Characteristics." In Int. J. LCA Vol. 5 (3): 143-144, Landsberg, Germany

Some new/old technologies in dairy processing

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Since the early part of 1900s, the dairy industry has seen great advances/ developments in the technology for the manufacture, for example, market milk (pasteurised, sterilized, extended shelf-life and UHT), butter fat separation and the processing of cream including the continuous process for butter making, evaporation and drying, cheesemaking, production of ice-cream membrane processing.

In general, such developments in dairy processing were achieved, as a consequence, advancements in automation and mechanization. It would be practically impossible to review developments in a short presentation; however, an overview regarding the latest technological developments regarding fermented milks including continuous production systems and packaging presented. Nevertheless, certain cheese varieties have seen great technological developments in mechanization and automation, whilst other varieties failed to accept parallel and similar developments - such aspects will be reviewed in terms of possible factors that may have influenced rejection of such technological developments. Similarly, the application/rejection of some membrane processing in some dairy products manufacture and on-farm handling of the milk also reviewed.

In conclusion, it is evident that mechanization/automation including continuous processes will be developed in the future to meet the demands of the dairy processors and the consumers, especially when health contributory aspects are taken into account. However, it is that such future advances will be influenced by existing statutory legislations and, furthermore developments in starter culture technology for the manufacture of wide range of fermented cheeses should not be overlooked.

New generation of starter cultures for continental cheese

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Starter cultures play a key role in the production of continental semi-hard cheese. Starter cultures in the continental cheese segment are usually composed of undefined mesophilic strains such as *Leuconostoc* spp. (*Leu.*) and *Lactococcus* spp. (*L.*). These cultures, termed LD cultures, play a significant role in flavour development and eye formation in continental cheese.

As cheese plants become larger and more effective there is an increasing demand for cultures developed to meet the requirements of the specific technology used in the dairy. Modern cheese culture suppliers will therefore aim at having a system employing state of the art strain differentiation and characterization at their disposal, which will be a valuable tool for a rapid identification of strains fitting to the individual needs of the cheese producers.

In close collaboration with cheese producers, Chr. Hansen has introduced a new generation of starter cultures for the Continental semi-hard cheese segment - the DCC cultures (Dutch Continental Cheese). The DCC cultures consist of multiple mixed cultures. Among these mainly highly active homofermentative (*L. lactis* subsp. *lactis*, *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar. *diacetyl/lactis*) and to a considerably less extent heterofermentative (*Leu. menesteroides* subsp. *cremoris*) strains blended with *Lactobacillus* and thermophilic *Streptococcus* strains.

During the presentation, tools for the development of new cultures and component thereof with help from the Chr. Hansen Global Strain Screening System (GSSS) will be presented. Furthermore, the main advantages of using F-DVS DCC cultures compared to traditional mesophilic LD cultures in semi-hard Continental cheese production will be claimed. Finally, present and future requirements for cultures to the continental semi-hard cheese segment will be discussed.

Milk delivery and processing in Slovenian dairies till 2002

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Slovene Dairy Association was established to achieve better communication between dairies, collection and exchange of information about milk quantities and processed dairy products, imports and exports and better communication with authorities. In the year 2002 the dairies received 473.5 million liters of milk, which was processed in different dairy products and sold on home market, as well as on markets in ex - Yugoslavia, EU, USA and Canada.

The total milk quantity produced and the one received in the dairies shows a constant growth of 3 % per year. Due to stimulate payment for "S grade" quality, there is a great improvement. Less than 50,000 mo/ml are now 90.78 % of all milk received in the dairies compared to 69.86 % in the year 1999. Improvement is in fat and protein %, although not so remarkable.

Increased milk quantity and stocks of products influenced the processing of the milk into more lasting products as milk powder, semi-hard cheeses, UHT products and butter.

In 2002 were exported dairy products in quantity app. 130 million liters of milk, which represented 27.5 % of all milk both by the dairies. Difficulties in exports are due to reciprocity agreements with some countries, lack of agreements with the others and restricted quantities and customs in EU and USA, respectively.

Milk solids future factor of new products

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Composition of cow's milk is very important for valorization of nutritional values of milk and for dairy industry because of decision about new products or using individual milk components in some new non-dairy products. Milk composition is in continually changes depend on race, feeding strategy and housing of cows. Some changes are slow till some are faster.

There is a whole line of components which become very important for all before mentioned categories of interests. Trends which will be further discern, and connected with components of dry matter, first are demands of consumers, and after that is introduction of new technologies.

Historically, dairy industry has passed through many changes. They have started on local level and today they happened in a big international companies and milk - which we are, at first, considered like nutritious liquid, and then like product with many important components, today it presents a base for many non-traditional dairy products, also like product with components which can be treated like - adding value by products of some industries. Development of technologies on more higher demands made possible identifying of new values like, for example nutraceuticals which become an interest to individuals studying human health, nutrition agriculture, food science and animal genomics.

It is sure that many of milk components, unknown and less important till yesterday, obtaining on their importance or through high number of researches about food and health characteristics of milk or like important components which will be used in future like components of other food.

This work will show some components and their importance.

The effect of n-3 Fatty acid supplementation on the milk and milk lipids Fatty acids composition in goat

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The effect of feeding supplements, rich in different nutritionally important fatty acids milk yield and composition was studied using 64 goats from 28 to 105 days after lambing. day adaptation period, the animals were divided into four groups. Goats were fed a control (Group CONT) or control diet supplemented with 20 g/day of: alpha linolenic (ALA), docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids for 5 days. Individual milk samples were collected during the 10 days adaptation and 5 days supplementation periods. In the 50-d lasting post-mentation period milk samples were collected first five days in daily intervals and afterward fifth day. Milk yield was recorded at each milking. Milk was analyzed for: fat, protein and fat composition. Statistical analysis showed no effect of FA supplementation on daily milk yield. Protein content decreased in time of supplementation and significantly increased (from 3.0 to 3.3 %, in Group CONT (from 3.0 to 3.05) during the supplementation period and 14 day after. While in the supplementation period no differences in milk protein content were observed, can't increase in protein content in Group ALA was observed during the post-supplementation. In the Group ALA milk fat content increased from 3.0 to 3.4 % during the supplementation. decrease of the milk fat content from 3.1 to 2.4 % was observed during the both periods. Acid composition was significantly altered by feeding of FA supplements. Depending on the added FA supplement the effects appeared different during the supplementation and post-mentation period. The addition of FA supplements decreased medium chain FA (C10-C16) from 45 % to 45 %, and monounsaturated FA (from 24 % to 22 %), but increased polyunsaturated FA (from 4 % to 11 %). In the adaptation period the n-3:n-6 FA ratio was 1:3.5, during the supplementation reduction to 1:1 in Group EPA, ALA and DHA, but not in Group CONT was observed. Efficiency of supplemented PUFA to milk fat was 13 % in Group ALA, 14 % in Group EPA and 15 % in Group DHA.

Preliminary investigation of the effect of somatic cell counts on physical and chemical characteristics of whole yoghurt*

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Mastitis is generally recognised as an important factor influencing the quality of milk and milk products. Milk from infected cows is characterised by increased somatic cell counts (SCC), which is correlated with changes in components and properties of raw milk. Previous studies indicated that high SCC can negatively affect the sensorial characteristics of plain yoghurt (2). The aim of the present study was to investigate the correlation between different bulk milk SCC and selected physical and chemical parameters of the resulting whole plain yoghurt. In order to achieve this aim, milk from individual cows was submitted to somatic cell counts (electronic counter, Bentley, USA) and classified in three groups based on individual SCC status: A - low SCC (<400,000 cells/mL), B - intermediate SCC (400,000-800,000 cells/mL), and C - high SCC (>800,000 cells/mL). Milk from the same group was bulked until a total of 50 L was obtained, standardised to 12.5 % (w/w) total solids content, and subsequently used for manufacture of stirred whole plain yoghurt. Yoghurts were stored in 1-L polyethylene bottles at 5 °C for 30 days. Yoghurt samples were collected and submitted to analysis at 1, 10, 20 and 30 days storage. Proteolysis was estimated using the concentrations of total nitrogen, non-protein nitrogen and non-casein nitrogen, according to AOAC (1). Lipolysis in yoghurt was expressed as free fat acid content - FFA (3). A rotational viscometer was used to evaluate the viscosity. Data were used to determine Pearson's Correlation Coefficient (*r*) between log SCC values and parameters studied. Student *t* test, with normal approximation, was used to compare correlation values at a 5 % level of significance. Preliminary results obtained after processing 4 replicate batches showed that although FFA content in yoghurt positively correlated with log SCC values, this fact was only significant (*P*<0.05) on day 1. This observation may be a consequence of greater lipolysis in fluid milk. Correlation between proteolysis and log SCC increased during 30 days of storage, yet results were not significant (*P*>0.05). Viscosity and log SCC values also showed a positive non-significant (*P*>0.05) relationship. Based on data obtained in the present investigation, it was concluded that high SCC values can increase lipid degradation in whole plain yoghurt.

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References

- Association of Official Analytical Chemists. 1990. *Official Methods of Analysis*. 15th Edition. AOAC, Arlington, VI.
- Oliveira, C.A.F., Fernandes A.M., Neto, O.C.C., Fonseca, L.F.L., Silva, E.O.T.R., Ballen, S.C. 2002. Composition and sensory evaluation of whole yoghurt produced from milk with different somatic cell counts. *Australian Journal of Dairy Technology*, 57: 192-196.
- Shipe, W.F., Senyk, G.F., Fountain, K.B. 1980. Modified copper soap solvent extraction for measuring free fatty acids in milk. *Journal of Dairy Science*, 63: 193-198.

Role of disinfection in premilking reduction of udder bacterial count

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Microbiologic quality of raw milk is the main indicator of its appropriateness for further processing into consumer milk and milk products. In milk production, animal health and optimal hygienic milking conditions are of crucial importance. The animals used for milk production must be regularly examined and healthy, thus the majority of bacteria reach milk via udder contamination during the process of milking. That is usually caused by inappropriate preparation of udder for milking. Therefore, the aim of the study was to assess the extent to which the introduction of disinfection in the udder premilking treatment reduced the udder bacterial count as compared with udder wiping with cloth and water. Two groups of 10 milk cows each from the same herd were included in the study. In group 1 animals, swabs were obtained from udder surface before and after usual premilking treatment by wiping with cloth and warm water. In group 2 animals, swabs were obtained before and after premilking treatment by wiping with cloth and warm water, followed by disinfection with a disinfectant based on surface active substances, organic acids and hydrogen peroxide (P3-Oxy Foam, Ecolab Co., Croatia). Milk samples for determination of total microorganism count were collected from both animal groups. Results of the study showed the total aerobic mesophilic bacterial count to be significantly reduced (*p*<0.05) irrespective of the method of udder premilking treatment. The total count of aerobic mesophilic bacteria decreased by 54.5 % after udder treatment with cloth and warm water, and by 88.1 % after udder treatment with disinfectant in addition to cloth and warm water. Comparison of the two techniques of udder premilking treatment also yielded a significant difference in the total count of aerobic mesophilic bacteria (*p*<0.05), whereby the additional procedure of disinfection decreased the bacterial count by 69.3 %. Also, the introduction of disinfection in udder preparation resulted in greater milk bacterial count decrease as compared with the standard procedure of udder wiping with cloth and warm water. Accordingly, premilking disinfection in addition to the usual udder washing reduced significantly the bacterial count on the udder skin, thus this method of sanitation can be recommended for use in the process of high-quality milk production intended for further processing.

Milk production and milk processing - veterinary legislation and official control

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Slovenian veterinary legislation governing the milk sector is contained in two regulations, being the *Rules on the veterinary-sanitary conditions for the production of foodstuffs of animal origin and placing on the market for public consumption* (UL RS 100/02) laying down the conditions to be met by establishments for the approval and registration, and the *Rules on the veterinary-sanitary inspection and control of the food production establishments, the veterinary-sanitary checks, and the conditions for the fitness for human consumption of foodstuffs and raw materials of animal origin* (UL RS 100/99, 38/00, 71/00) laying down the inspection and control and conditions for the health suitability of all foodstuffs of animal origin. The *acquis* regulates each type of foodstuffs of animal origin in a separate regulation, by the vertical directives (milk is regulated in Directive 92/46/EEC). The requirements of this Directive are included in both our regulations (Rules) referred to above.

The basic objective of legislation is to ensure food safety to consumers. The producers' own checks and their responsibility to place on the market foodstuffs only that are fit for human consumption represent the basis of the "from stable to table" concept, and the HACCP system represents the basic principle of providing safe food. In Slovenian dairy industry, the HACCP system is not a new concept anymore as the dairy establishments were the first from among the food production establishments to introduce it. The basis of HACCP is the good hygiene practice (provision of clean potable water, the hygiene of premises and equipment so as to avoid the contamination of products, good cleaning and disinfection practices, personal hygiene and good health of operators, production procedures designed so as to avoid food contamination, etc.), which is prescribed in the EU Milk Directive as well as in the Slovenian veterinary legislation. Sectoral Codes of Hygiene or the Good Hygiene Practice Codes, envisaged also in the horizontal Directive on foodstuffs (93/43/EEC), are in many Member States one of the priority tasks of sectoral associations (chambers) and officially confirmed by the competent authorities. In Belgium, the so-called System of total milk quality assurance ("IKM- QFL-QMK") was developed on the basis of such sectoral initiative, which begins at a dairy farm.

Official inspection and control in the milk sector in Slovenia is carried out by the veterinary inspection services in milk production and processing, and by the public health inspection services in the trade (their respective competence is laid down in the Foodstuffs Act). Legal basis for the carrying out of veterinary inspection and control is provided in the *Rules on the veterinary-sanitary inspection and control of the food production establishments, the veterinary-sanitary checks, and the conditions for the fitness for human consumption of foodstuffs and raw materials of animal origin, transposing inter alia* also Directive 96/23/EC and Decision 97/1747/EEC laying down the control of residues of harmful substances in foodstuffs.

Highpressure treatment of milk: effects on surface hydrophobicity of milk proteins

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High-pressure (HP) treatment is an emerging technology that is being developed and is a minimal process for the production of a wide variety of safe and nutritious foods. Of particular importance in the production of dairy products is the modification of protein structure and properties resulting from HP treatment.

In this respect, we have analyzed the effect of HP treatment (110 to 440 MPa at 25 °C for 10 and 20 min.) on protein structure and casein micelle surface hydrophobicity of UHT skim (0.1 % fat), 1.5 % fat and whole (3.2 % fat) milk. The surface hydrophobicity is studied via spin titration of a fluorescence marker (ANS). The data has been analyzed with Michaelis-Menten model with F_{max} (the number of hydrophobic portions localized on the surface), $1/K_d$ (the binding affinity of ANS to milk protein) and F_{max} / K_d (the strength of binding).

The results have revealed that as HP increases from 110 to 220 to 330 and finally to 440 MPa, F_{max} also increased from 60 to 70 to 80 and 90 A.U. respectively in UHT whole milk. The effect of fat has also found to be effective on hydrophobicity as F_{max} values decreased with increasing time at all the pressure-time-temperature combinations studied. However, the effect of pressure (10 and 20 min.) showed an increase up to 220 MPa with a drastic decrease thereafter. The affinity of ANS for binding to milk proteins seem to be unaffected with HP treatment with conditions studied but binding strength of ANS showed gradual increase with increasing pressure. This fierce binding of ANS to hydrophobic surfaces on milk protein indicates that these hydrophobic areas are ready for other possible reactions.

In the light of these results, it can be concluded that HP has an effect on non-covalent bonds in milk and especially hydrophobic bonds. With the effect of increasing pressure, the proteins possibly decompose into sub-micelles and the imbedded hydrophobic areas inside these micelles position in such a way that they can readily interfere with the fluorescent marker, ANS.

Chemical properties of goat milk

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The chemical composition of goat milk was investigated. The studies were performed on 912 individual milk samples collected from goats of alpine and saanen breed. The average quantity of fat in examined samples was 3.46 % (2.23-6.07 %), proteins 2.66 % (1.87-4.63 %), lactose 4.80 % (3.43-5.55 %), non-fat dry matter 8.24 % (7.04-10.02 %), respectively, the content of dry matter was on an average 11.70 % (9.27-16.90 %), and the content of water 88.30 % (83.10-90.73 %), respectively. During 7 lactation periods, the greatest variations were recorded in fat content and minimum in protein and lactose content.

Key words: goat milk, chemical properties

Evaluation of plain yoghurt manufactured from buffalo milk with different fat content*

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Yoghurt is considered an important dairy product for human nutrition. In Brazil, it is traditionally manufactured from cow's milk. Yoghurt can be also an excellent alternative for utilisation of buffalo milk. However, consumers may have some acceptance problems in relation to buffalo yoghurt because of the original milk high fat content. The aim of the present study was to evaluate some characteristics and the stability of yoghurt produced with buffalo milk standardised at different fat levels. In order to achieve this aim, milk was defatted down up 0.5 % and mixed with adequate volumes of whole milk, resulting in three treatments: A- whole yoghurt, B- standard yoghurt with 3.5 % fat content, and C- skim yoghurt with 0.5 % fat content. Standardised milks were used separately for manufacture of stirred whole plain yoghurt. Yoghurts were stored in 1-L polyethylene bottles at 5 °C for 30 days. Samples were collected at 1, 15 and 30 days of storage and submitted to the following analysis: coliform, mesophile and psychotropic counts (1), determination of pH, fat and protein content, total solids and non-fat solids (2), viscosity and sensorial evaluation (3). Results were analysed using one-way ANOVA. Microbiological results showed no significant differences ($P>0.05$) between yoghurts produced from whole, standardised and skim buffalo milk. No important variations in chemical parameters were observed during 30 days storage of all yoghurts. Viscosity was not influenced ($P>0.05$) by fat content in yoghurts on days 1, 15 and 30 after manufacture, hence indicating that addition of milk powder is not necessary to standardise solid contents in yoghurt made from defatted buffalo milk. Standardised yoghurt obtained significantly ($P<0.05$) higher grades in sensorial analysis at 15 and 30 days storage when compared to yoghurt produced from skim milk. It was concluded that all yoghurts presented acceptable microbiological, physical and chemical characteristics. However, the product made from buffalo milk containing 3.5 % fat showed better sensorial quality and good stability during storage.

* Trial financially supported by FAPESP - grant nº 2001/03817-0.

References

1. American Public Health Association, 1992, *Standard methods for the examination of dairy products*, 16th ed. Washington: APHA.
2. Association of Official Analytical Chemists, 1990, *Official Methods of Analysis*, 15th Edition. AOAC, Arlington, VI.
3. Institute of Food Technologists, 1981, Sensory evaluation guide for testing food and beverage products. *Food Technology*, 35: 50-59.

The effect of the curd treatment onto the quality of the Trappist cheese

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The Trappist cheese is a world-famous cheese, originating from the monasteries in France. It is very popular on the world market, because of its characteristic trade's thanks to which; it is recognizable no matter where it is being produced. This cheese has a marvellous, specifically mild flavour and pleasant smell. Cheese textured is light yellow, elastic, with regularly arranged punctures of a diameter in between 5 and 10 millimetres.

The curd treatment is the most delicate phase in the technological process of the Trappist cheese production. Although, in comparison to the whole process, this particular phase lasts very short (2-3 hours), it is the basis for further processing, on which the ultimate success of the final product mostly depends. This all the operations must be performed very carefully and specific set of rules must be obeyed. The governing aim of the curd treatment is to adjust the percentage of moisture by bringing it to the necessary level; especially because of the fact the Trappist cheese has a soft consistency.

Since the creation of the cheese grain is considered to be the essential operation on which the quality of the cheese depends, in our research we dedicated special attention to the content of the cheese grain, to its size, and role in controlling the percentage of the moisture present in the cheese.

The Trappist cheese has relatively moist dough, therefore the cheese grain must not be too drained. What is required is to achieve a specific firmness and to provide the conditions for microbiological processes and the biochemical transformation of certain milk ingredients into cheese.

Since the section of cheese represents a very important characteristic which influences the potential consumer, our research is focused on the technological achievements in order to establish the conditions for obtaining the typical section as a mark of a standard quality.

Chemical composition of milk of two grazing dairy goat breeds

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The survey was carried out on 10 goats of Alpine and 10 goats of Saanen breed. Feeded exclusively green forage in mountain pastures at the altitude of 623 m and 1077 m, respectively animals were in excellent health. Four samples per goat were collected in the middle of the in September.

Basic chemical composition was determined immediately (some hours after milking), analyses for milk fat composition and free fatty acids (FFA) were deep frozen at -18 °C and at -7 °C, respectively. Analyses were performed within one week. Gerber method for fat determination method described by Deeth et al. (1) for FFA were used. Milk fat composition analyses were conducted by *in situ* transesterification and capillary column gas-liquid chromatography.

The average milk composition of Saanen breed was 3.77 % fat, 3.4 % total protein, 4.31 % lactose and 12.26 total solids. The average milk composition of Alpine breed was 3.39 % fat, 2.9 % protein, 4.02 % lactose and 11.06 % total solids. Mean value of the FFA was 1.02 mmol/l in Saanen breed milk, and 0.85 mmol/l in the milk of Alpine breed, the difference was statistically significant ($P > 0.001$). There was a positive correlation between the FFA and fat content ($r = 0.73$; $P = 0.001$). Average content of saturated fatty acids in milk was 74.52 % and 73.05 % for Saanen and Alpine breed, respectively. Monounsaturated fatty acids were more abundant in milk from Alpine (20.49 %) than in milk from Saanen breed (20.49 %).

The average content of the polyunsaturated fatty acids was the highest in milk fat from Alpine breed (3.73 %) compared to Saanen breed (3.24 %). Three saturated fatty acids (capric acid (C10), myristic acid (C12) and palmitic acid (C16)), were present at significantly higher levels in samples from the Saanen breed than those from the Alpine breed. Among monounsaturated fatty acids stearic + palmitoleic acid (16:1, n-7) showed significantly higher concentration in milk samples from Alpine breed than in milk samples from Saanen breed. The content of linoleic+linolenic acid (18:2, n-6) was significantly higher in comparison to milk samples from Saanen breed. Between the proportions of other fatty acids (8:0, 14:0, 15:0 iso, 15:0 anteiso, 17:0 iso, 17:0 anteiso, 18:1, n-7, 18:1, n-3 and 20:0) present in milk fat of Alpine and Saanen breed the differences were statistically significant.

References

Deeth, H.C./Fitz-Gerald, C.H. Lipolytic enzymes and hydrolytic rancidity in milk and milk products. V. Developments in dairy-2 (ed. Fox P.F). London, Applied science publishers, 1983, s. 195-227

Abamectin in blood plasma and milk of lactating dairy sheep

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The aim of the present study was to estimate permeation of abamectin (ABM) into sheep's milk after subcutaneous administration by following its time course in blood plasma and milk. ABM is an endectocide with a broad spectrum of activity against nematode and arthropod parasites of animals and plants. In Slovenia as in EU countries it is banned for use in animals from which milk is produced for human consumption.

The experiment was performed on six Istrian Pramenka sheep in the early lactating period. They were administered a single subcutaneous dose of 0.2 mg/kg b.w. of ABM (Abamitel®, Krka d.d., Novo mesto, Slovenia). Individual blood plasma and milk samples were taken from day 1 to day 42 after treatment. Analysis was performed by SPE clean-up, pre-column derivatisation and HPLC-fluorescence detection of the B_{1a} component of ABM in collected samples. The limit of detection was 0.04 µgB_{1a}/l for both matrices. Maximum concentration C_{max} and time to maximum concentration t_{max} were reported as observed. ABM pharmacokinetics was assessed by a one-compartment model.

Individual C_{max} in the sheep's blood plasma and milk was 42.0 and 37.5 µgB_{1a}/l, respectively. Mean C_{max}, t_{max}, t_{1/2ke} and MRT for plasma data were: 30.9 µgB_{1a}/l, 2 days, 1.7 days and 3.7 days, respectively, and for milk: 26.8 µgB_{1a}/l, 2 days, 1.9 days and 3.8 days, respectively. Mean time in which concentration fell below limit of detection was 23.5 days for blood plasma and 23 days for milk. Milk to plasma concentration ratio of 0.89 was estimated by comparing milk and plasma AUC values.

As ABM use during lactation is prohibited, the presence of ABM residues in milk merits attention. The time for ABM residues to fall below the limit of detection was long and was similar to ivermectin. However, comparison of milk to plasma concentration ratios indicates that extent of ABM permeation into milk is lower. The results reported contribute to the further understanding of the ABM excretion patterns in lactating sheep.

Oscillation in the quality and composition of purchased milk in Slovenia in the period from 1995 to 2002

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In Slovenia the amount of purchased milk in the studied period increased from 383 mill. l of milk in 1996 to 473.5 mill. l of milk in the year 2002. Every month about 30 to 45 mill. l of milk was purchased. During the year the amounts of purchased milk varied, therefore most of milk was purchased in May and the least in February. Some great changes were noticed also in number of farms that sold milk to dairies. In 1995 30,000 farms sold milk to dairies and in 2002 only 12,500 farms. The number of cows that produced milk for sale diminished from 132,532 in the year 1995 to 113,599 in 2002. Hence the amount of purchased milk increased per cow (2,986 l compared to 4,154 l) and per farm (12,942 l compared to 38,577 l). The share of farms rearing one to four cows diminished (62 % in 1995 compared to 36 % in the year 2002) while the share of farms that reared above 10 dairy cows increased (9.3 % in the year 1995 compared to 30 % in the year 2002). The average number of dairy cows per herd increased from 4.4 in the year 1995 to 9.3 cows in 2002. Great changes were noticed also in milk composition and quality of purchased milk. The average content of fat in milk purchased by Slovenian dairies was 3.93 % in 1995 compared to 4.13 % in the year 2002. A similar content although a bit lower was noticed in proteins. The average content of proteins increased from 3.24 % in 1995 to 3.36 % in the year 2000. During the year seasonal oscillations were noticed regarding the content of fat and proteins. Milk contained low amounts of fat and proteins in summer months (June, July, August) and the highest ones in late autumn and winter (November, December, January). The difference between the highest average content of fat ranged between 0.26 % in 1996 and 0.44 % in the year 2002. The differences in protein contents were a bit lower and ranged between 0.16 % in the years 2000 and 2002 and 0.24 % in the years 1996 and 1998. More attention has been paid to microbiological quality of purchased milk since 1993 when Ministry of Agriculture, Forestry and Nutrition introduced Regulation according which the quality of milk was rewarded and milk containing too many micro-organisms deducted. A percentage of milk with up to 100,000 m.o./ml milk increased to reach over 97 % of all purchased milk in the year 2002. It means that only 3 % of purchased milk does not agree with severe European quality measurements for purchased milk. About 91 % of purchased milk contained less than 50,000 m.o./ml of milk in the year 2002 and only 61.5 % of milk in the year 1995. Such good quality of milk is rewarded by 5 % extra payments. The quality of milk for consumption and processing depends also on total somatic cell count (SCC) in milk. In the year 1996 the conditions for SCC in milk became more severe. Financial rewards and advising service contributed to better quality of milk regarding the SCC. In 1996 only 75 % of purchased milk contained less than 400 thousand somatic cells in ml of milk and in the year 2002 about 93 %.

Key words: dairy cows, purchased milk, composition of milk, quality of milk

Use of adjunct starters to improve the quality of Emmental cheese

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In case of hard cheeses made from raw milk, the development of characteristic features during maturation highly depends on the metabolic activities of the added starters, but also on the indigenous microflora of the raw milk, particularly the non-starter lactic acid bacteria (NSLAB). A number of attempts have been made to isolate facultatively heterofermentative lactobacilli (FHL) from cheese for using them as adjunct starters in order to improve quality attributes such as appearance and aroma, to avoid cheese defects such as excessive secondary fermentation, or to inhibit excessive growth of undesired enterococci.

Out of more than 200 isolates from the cheese-making environment, a number of FHL strains (*Lactobacillus casei* subsp. *casei*, *L. rhamnosus*) were selected with respect to their autolysis properties, as well as with respect to their ability to metabolise citric acid and ribose. These FHL were added as adjunct starters during the production of Emmental cheeses in pilot-plant scale. Cheese microbiology as well as chemical and biochemical parameters were monitored throughout ripening, and mature cheeses were subjected to mechanical and sensory analysis.

In control cheeses (without adjuncts) from raw milk, the FHL reached a maximum of 10^8 cfu/g within 4 weeks, and mature cheeses contained 10^7 cfu/g. When adding three *L. casei* subsp. *casei* adjunct starters, which varied in their susceptibility towards autolysis, to raw and pasteurised milk we observed similar FHL counts. In control Emmental from pasteurised milk, FHL were not detected. Propionibacteria counts and propionic acid contents revealed a significantly reduced intensity of the propionic acid fermentation for cheeses made with FHL adjuncts. Propionic acid content was 102 mmol/kg and 73 mmol/kg for cheeses made without and with FHL starters. Whereas the citric acid content in control cheeses remained constant throughout maturation, citric acid was almost completely metabolised by the FHL adjuncts, suggesting that FHL and propionibacteria compete for identical nutrients, which can be utilised more efficiently by the FHL. Whereas enterococci counts increased from approximately 10^4 cfu/g to 10^6 cfu/g during maturation of raw milk control cheeses, the growth of enterococci was significantly reduced in raw milk Emmental made with FHL adjuncts. This indicates that FHL autolysis and the related intensity of the liberation of intracellular enzymes can be used as an indicator for the suppressive effect of adjuncts towards the development of enterococci.

When adding FHL strains with different capability of utilising citric acid and ribose, a trend towards an increased inhibition of enterococci was observed when using *L. rhamnosus* strains. The addition of citrate (+) adjunct starters resulted in a complete degradation of citric acid, and a reduced evolution of propionic acid during ripening. Both texture and sensory assessments resulted in insignificant differences, implying that the FHL do not show any negative effects on the organoleptic properties of the cheeses.

Whey - from waste to gold

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In the past, whey was used as a secondary product, and was not further processed. It was considered waste, which was simply disposed into the rivers or in best cases used as a food for pigs. Today, thanks to the development of new technologies like ultrafiltration and nanofiltration, whey offers almost unlimited uses. Its nutritional value is becoming more known: high amount of lactose, which is necessary for development of nerve tissue, minerals (potassium, phosphorus, & sodium), high-value proteins like lactalbumin and lactoglobulin. Recently research of proteins (especially immunoglobulin, lactoferrin and (-lactoglobulin) has shown their ability to stop the spread of colon cancer. Even more whey is processed into quality protein concentrates, lactose and powdered whey, which is used in all branches of food industry. Importance of whey processing to the dairy industry is high and therefore in this project assignment we have concentrated on the processing options, ecological and financial problems and whey related costs and benefits, as we believe it shouldn't be neglected.

Keywords: whey, Whey Protein Concentrate (WPC), lactose, ultra filtration, functional food, dietary protein, bioactive peptides

Rheological evaluation of dairy products

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When focusing on rheological properties of foods and, in our case, dairy products, one has to consider a number of different aspects. First of all, but also depending on the product under consideration, rheology and texture may largely determine sensory properties and hedonic quality. Independent whether a particular products macroscopically appears as 'solid' or as a 'liquid', rheology is one of the main factors determining essential processing steps such as transportation through pumping, size reduction, or forming. Finally, both sensory properties and processing issues are related to food structure, which can be viewed at largely differing levels of magnification. The use of ingredients with specific functional properties and the application of tailored processing steps or, more simply, structure engineering, gains increasing importance in the development and the marketing of dairy or dairy-based foods.

As regards the equipment available for rheological analysis but also reference works in the literature, a tremendous progress was made within the past decade. Commercially available instruments are said to be less expensive, more accurate and reliable and easier to maintain, and information is widespread along scientific journals and books. However, we should also consider some of the disadvantages of this progress, which is not that easy to extract from the available sources:

- ▶ We are aware of commercially available instruments with unjustified specifications: firmness testing easily becomes increasingly erroneous when the gap between product firmness and instrument stiffness diminishes;
- ▶ We are aware of standardised methods with a very limited scope: non-consideration of the structure of the material under evaluation and careless interpretation of the may result in deficient conclusions;
- ▶ We are aware of electronic tools allowing preposterous calculations: software is often overloaded with numerical or statistical helpers, and it is not always easy to identify their drawbacks; and, finally
- ▶ We are aware of products which resist very effectively against rheological analysis: different operating principles or small changes in test setup sometimes show large effects on the response data, thus only leading to unjustified results with a highly relative character.

Although it is hardly impossible to propose a full itemised 'to-do-list' for a good practise in a rheological laboratory, some basic hints for achieving reliable results can be extracted from the above listing. These include a proper maintenance, calibration and control of the equipment, a fundamental knowledge of boundary conditions related to the product under study, and a critical evaluation and a very careful approach towards the measured data. Only if these items are fulfilled, reasonable and reliable data will be obtained.

Oscillation in somatic cell count and in basic milk substances in AP control cows in Slovenia in the period from 1995 to 2002

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The variability of milk substances in AP control cows in Slovenia was studied between the years 1995 and 2002. Statistical analysis included 4,078,286 monthly measurements of AP control for quantity and composition of milk and 1,490,252 measurements for somatic cell count (SCC) in milk of individual cows. In the population of AP control cows Simmental cows prevailed (41.6 %) followed by Black/White (34.6 %) and Brown ones (23.8 %). The highest milk production was achieved by Black/White cows (20.9 kg/MD) and the lowest by Simmental cows (14.3 kg/MD). Milk composition differed among breeds as well. The highest content of fat on the control day was found in Simmental cows (4.27 %) and the lowest in Black/White cows (4.18 %). Similar results were achieved for protein content being the highest in Simmental cows (3.44 %), followed by Brown breed (3.41 %) and the Black/White one (3.37 %). Milk production tended to be higher in that time on the control day in all breeds but the milk production increased the most in Black/White cows and the least in Brown ones. The average content of fat in the three breeds increased from 4.06 % in 1995 to 4.30 % in 2001. The average content of fat increased the most in Simmental cows (4.04 % in 1995 compared to 4.36 % in 2001). A similar increase was found in proteins: 3.26 % compared to 3.46 % in the last two years. The analysis of results showed seasonal oscillation in milk production as well as in milk composition of AP control cows. The highest milk production was noticed in spring and summer months, and the lowest in late autumn and winter but just the opposite results were achieved for milk composition that is, milk was the richest in winter and in late autumn when milk production was lower and the poorest in summer months when the milk production was the highest. The lower content of fat and proteins in summer months is the result of nutritional regime and high temperatures as well as unpleasant climate, which is evident in higher SCC in milk and more frequent udder difficulties. It was found out that the average SCC was the highest in Black/White cows (477×10^3 SCC/ml milk) and the lowest in Simmental cows (324×10^3 SCC/ml milk). SCC in milk has been a criterion for milk payments since 1996. It was also determined that SCC in all three breeds decreased and udder conditions improved, which was the result of better herd management and breeders' awareness. Most of AP control results (90.3 %) were obtained on private farms where a bit lower milk production was noticed but higher contents of milk substances as well as lower SCC compared to cows on ex-state farms. The analysis of monthly measurements of AP control showed that 23.7 % of AP control cows had over 400 thousand SCC/ml milk and only 65.0 % cows up to 250 thousand SCC/ml milk. The average milk production in standard lactation increased for 1,056 kg milk in the observed period (4,505 kg in 1995 compared to 5,561 kg in 2002). The difference in milk production between Black/White cows and Simmental ones was about 2,200 kg in the lactation period.

Key words: cattle, production control, milk production, milk substances, SCC

b

Food Safety -
Risks and
Quality Assurance -
New Analytical Tools

Diagnostical use of MILK laboratory proficiency testing

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I) The composition of raw milk (RM) is important for milk recording and for quality evaluation. The reliability of routine analytical results is therefore significant. A mistake occurrence could jeopardize the efficiency of results. Czech milk laboratories (ML) carry out accreditation (EN ISO/IEC 17025) - perform the systems of quality assurance of the milk analysators. Systems are improved by projects QF 3019 and LA103 solution. II) Czech routine ML (milk recording ML = individual milk samples; central ML = bulk milk samples) take part in the proficiency testing (PT). RICH Rapotín organizes PT of the milk recording ML for some RM components: fat (F); protein (P); lactose (L); urea (U). Tests are carried out by 10 RM samples with modified variability of components. At evaluation of PT results is used system of the Euclidian distance from the origin (Re; LERAY, 1993) for participants ordering by their milk analytical results accuracy. III) Two types (designs) of systematic diagnostical graphs were constructed on the Re basis. First type (design) of diagnostical diagram for RM analysators is based on Shewhart's diagram principles for Re parameter. The graphical historical discrimination limits of Re (HDLRes) were calculated for F, P, L and U according to long-time result rows (2 years = 22 tests; as robust median-quartile estimation of confidence interval at probability level 95 %). Second type (design) is based on analysator set comparisons. The diagnostical graphs (both designs) for individual instruments and milk analytical parameters (1) and for set of instruments and milk analytical parameters (2) are accessible for workers of ML: 1) Re situations before and after calibration are shown by the alternating system of signs (OxOx...) in the row of calibrations and proficiency testings. It makes possible the diagnosis and incidental co-ordinating of the corrections in the case of the unconformity occurrence. It is possible to show separately two components of the Re: mean difference (d); variability of differences (sd); by the same type of graph. It improves the diagnosis of incidental occurrence of the result mistake in terms of effect estimation of systematical and random mistake components on total unconformity value; 2) modified system shows the situations of analysator set by Re (Re=(d²+sd²)^{0.5}; original Re rendering, LERAY, 1993) by comparison of two graphs after last calibration and before next calibration (xO). The opposite rendering is possible as well - in two comparison graphs in one design and in two design types (after and before calibration and before and after calibration of analysator set). It improves the diagnostical possibilities for incidental unconformities as well. For instance it is possible to demonstrate four types of existed instruments for good imagination of diagnostical ability of graphs: A) master instrument in ML system; B) routine instrument (RI) - good stability; C) RI - normal stability; D) RI - unconformity occurrence. IV) The positive contributions of the developed diagnostical graphical system are expected to: - improvement of estimation of unconformity sources of RM routine analytical results, their origin and character; - determination and improvement of incidental result corrections and instrument repairs; - improvement of reliability of routine results of RM analyses in general.

Detection and enumeration of probiotic microorganisms in food and feed

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Introduction and scope

Although the application of advanced methods has revolutionized the insight into the nature of lactic acid bacteria contained in food and feed, the reliable detection and enumeration of these micro-organisms has still remained an area of challenge. Today, the knowledge about detailed microbiological composition of fermented dairy products is of interest for two reasons: on one hand, criteria such as brand name, authenticity and classification of the product are strongly based on the presence of certain micro-organisms (Genus and Species) at certain numbers (for example see legal hurdles for yogurt), on the other hand, bio-functional (e.g. probiotic) effects are known to depend on the presence of certain bacterial strains, also at certain numbers and under viable conditions.

During the last decade, several methods have been described and evaluated in the literature offering some usefulness in terms of selective detection and enumeration. However, the more insight views are gained the more drawbacks are getting evident with some of the techniques described. Problems encountered relate, e.g., to time-consuming media preparation, availability of ingredients and - last but not least - to insufficient selectivity properties of the media under practical conditions.

Ongoing developments

Although a variety of methodological techniques ranging from culture methods, over biochemical, up to genotypic confirmation are necessary to comprehensively examine the microbiological composition in probiotic foods and feeds, simple and selective culture methods are of primary relevance for routine purposes. At present a group of experts coordinated by the International Dairy Federation is dealing with the optimisation and the development of standardised methods for the enumeration of bifidobacteria and Lactobacillus acidophilus. According to results obtained in recently performed collaborative tests, an agar medium supplemented with mupirocin seems to fulfil the requirements necessary for a reliable detection and enumeration of bifidobacteria. As far as the assessment of Lactobacillus acidophilus is concerned, it is not clarified yet whether the use of MRS medium supplemented with clindamycin is of advantage over other techniques. In the light of the emerging multitude of products containing a mixed microbial population (e.g., *L. acidophilus*, *L. casei*, *L. rhamnosus*, bifidobacteria, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*) it will be necessary to clearly discriminate among the diverse components.

Aims and conclusions

In this lecture, examples and suggestions for possible methodological strategies for the examination of probiotic micro-organisms in food and feed will be presented.

Raw milk analysis - scope and quality assurance aspects

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There is a wide use of raw milk analysis for different purposes since a long time. Over the years ago, emphasis was laid on detecting falsification of milk by adding water and on neutral basis for fair payment between dairy farmer and milk trader. Soon raw milk analysis played an important role in dairy herd improvement schemes, too.

During the last decades raw milk analysis found its central position for the different quality assurance (QA) schemes working in the dairy food chain. Linking milk payment to several parameters describing the production value of raw milk was a key to the high level of efficiency and productivity performed in dairy business today at the same time. Total bacteria count, somatic cell count testing for inhibitors are the basis to provide best raw material to the dairy industry.

Consumers in Europe have developed a differentiated assessment to food quality during the last decades. Quality cannot be described by mere physical properties any longer. Since it has become a matter of course that physical quality is best / premium at any time and at any place called "soft" quality parameters (mode of production, ethical aspects, emotional added value) gained more and more importance in the markets. Nevertheless raw milk analysis still is the safe and trustworthy dairy products. With the ongoing integration of all partners of the dairy chain and the need to set up systems for tracing back food to the origin, raw milk analysis will become more and more a central source for relevant information.

Quality assurance at different levels and from different points of view is very necessary for the process of integration of all partners and stakeholders over the whole dairy food chain in a system of trustful cooperation. Analytical aspects in raw milk are in the focus of this lecture along the chain from sampling, transport, processing in the automated raw milk laboratory, consolidation and delivering the data is one topic. QA for labs as an "organism" and between related to different reference methods - or better - reference systems is another very important topic. Reference methods are only as good as the ability to provide the obtained data to calibration performance testing routine procedures. Mainly this is done with reference or secondary reference materials (SRMs). QA at this level should deal with reference systems integrating all factors involved in the production of raw milk.

New ways of producing SRMs as deep frozen raw milk samples are described and an example for a reference system is given. A discussion should be initiated, how to synchronize internal bodies in their efforts to create reliable QA systems and linking all relevant activities on a world scale. Raw milk analysis can provide a very broad basis for a world wide QA system of milk and products, on which food safety and traceability issues can be grounded.

Probiotics, prebiotics and natural components in nutrition of dairy animals

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Probiotics are biopreparations containing living cells or metabolites of stabilized autochthonous microorganisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and the immunity of the host. These preparations are used in farm-animal nutrition for feed preservation, improving the conversion of feed nutrients, and improving production, in the manipulation of the functional development of the digestive tract of young animals, and in the prevention and therapy of diseases in humans, and farm animals. The mode of inhibitive action of probiotics against pathogens may be mediated by competition for receptors on the gut mucosa, competition for nutrients, the production of antibacterial substances, and the stimulation of immunity. Probiotics influence digestive processes by the improvement of the microbial population that is beneficial for the macroorganism, by enhancing its enzyme activity, by improving digestibility and feed utilization. The optimization of digestion can be exhibited by growth, stimulative effect, and higher weight gains. Despite a lot of knowledge obtained, the mode of action of probiotics has not been explained yet. In order to enhance the efficacy of probiotics, it is necessary to obtain additional important knowledge on the mechanisms mediating their effect in the digestive tract. The enhancement of the efficacy of probiotics by their combination with synergistic acting components is frequently used in practice. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. A way of potentiating the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics which may be defined as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by activating the metabolism of one or a limited number of health-promoting bacteria and/or by selectively stimulating their growth and improving the hosts welfare. Synbiotics seem to be preparations whose potentiated protective and stimulative effects occur only in the colon. Taking into account the pathogenesis of diarrhoeic diseases in young animals there is a need for protecting the digestive tract mucosa throughout its length, i.e. also in the small intestine so that the adhesion of pathogenic microorganisms can be prevented. In various disorders of the gastro-intestinal tract, there occur conditions for the translocation of conditioned pathogens or pathogens from the colon into the front part of the digestive tract. Potentiated probiotics are defined as biopreparations containing production strains of microorganisms and synergistically acting components of natural origin potentiate their probiotic effect on both small intestine and colon and their beneficial effect on the host by intensifying a mechanism or by extending the range of their probiotic action. It is suggested that combining probiotic microorganisms with specific and nonspecific substrates, metabolic products of microorganisms, plant extracts and trace elements could improve their positive effect on the host.

Bacteriocins, enterocins and their antimicrobial effect in dairy products

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Production of high-quality products is important for both, dairy companies as well as for consumers. Dairy companies must gain and maintain consumer loyalty and consumers request safe products. The loss of products due to spoilage before consumption represents lost profits. The other important aspect is health of consumers. The presence of high numbers of bacteria in the raw milk as well as in pasteurized milk causes decrease of the products quality. Therefore, preventing as well as reducing factors are searched to prevent and/or to reduce contaminants without influence on sensory quality of the products. Bacteriocins represent one group of those substances. Moreover, some of bacteriocins- enterocin-producing strains including have also probiotic characters. That is, the aim of this study was to check the effect of enterocins CCM4231, *ent* A, *ent* M (produced by our strains *Enterococcus faecium* CCM4231, EK13 and AL41) in different dairy products against *Staphylococcus aureus*, *S. vitulinus*, *Listeria monocytogenes*, *L. innocua* by which the products were experimentally contaminated during processing or during their storage. After 24 h a reduction 1.5 and 1.6 log cycles was noted in goat and cow cheese lump against *S. aureus* SA5 with prolonged influence up to 168 h (2.4; 2.5 log cycles) by *ent* A and *ent* M; while activity of *ent* A caused a difference 4.9 log cycles up to 168 h in goat cheese lump against *S. vitulinus*. Reductive effect of *ent* CCM4231 against *L. innocua* or *L. monocytogenes* and *S. aureus* Oxford 209P was also found in cheese, bryndza cheese and yogurt. Sensory quality as well as pH of the products was not influenced; that is, real possibilities to use bacteriocins-enterocins in dairy products as protective components are indicated. However, legislation is problem for discussion.

The results were founded by the project VEGA 2/2043/23.

Demonstration of probiotic properties - review with an emphasis on our own experiences in testing two *Lactobacillus gasseri* strains

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Lactic acid bacteria, especially lactobacilli are considered the most common and also promising probiotics, namely bacteria that are able to exert defined positive functional effects when consumed alive in a sufficient amount and thus promoting intestinal balance and health. These functional effects can be established *in vitro* by the use of probiotic bacteria cultures and cultivated intestinal cells, *ex vivo* on intestinal tissue, and *in vivo* on animal models, in human clinical trials or nutrition studies.

In the present contribution, different steps of functional testing of probiotics are presented and illustrated with the results obtained during our own research work on two *Lactobacillus (Lb.) gasseri* strains, isolates from human faeces. Among basic probiotic properties, the survival under conditions in GI tract, production of metabolites that are able to inhibit undesired or pathogenic bacteria, ability to adhere to cultured intestinal enterocytes and on the intestinal mucosa, and sensitivity to antibiotics are pointed out. The study of competitive exclusion as one of the mechanisms involved in antagonistic activity is mentioned and presented by the results obtained with *Lb. gasseri* and *E. coli* O8K88H9 *ent⁺* *ex vivo* on intestinal mucosa of piglets and in gnotobiotic piglets. The *ex vivo* adhesion trials on the mucosa of pigs' small intestines showed adhesion of *Lb. gasseri* strains, while competitive exclusion of *E. coli* cells was not observed at the concentrations tested and under the conditions applied. However, beside production of organic acids and stimulation of immune response, the competitive exclusion was probably involved in the inhibition of *E. coli* by *L. gasseri* K7 in *in vivo* trial on artificially infected gnotobiotic piglets. Further, stimulation of immune response by probiotic bacteria which has been demonstrated in many studies performed *in vitro* on cultured cell lines, *in vivo* in mice, piglets and other animal models, or in human trials, is exposed as one of the most important features. We should take into consideration that some of desirable properties such as the origin of the strain from the same species as it is intended to be used for, may not be so important as was considered previously; for example, it was shown recently that the strains of human origin can adhere successfully to animal mucus and vice versa. On the other hand, the good results obtained *in vitro* may not guarantee good performance *in vivo*. Therefore, the *in vivo* testing as a final step to confirm the potential health effects is necessary.

As the meaning of technological features should not be underestimated, a considerable part is devoted to the presentation of evaluation of different modes of preparation of *L. gasseri* test strains for probiotic application, such as freeze drying, incorporation of test strains in cheese or in fermented milk, or freeze drying in different media. When included in fermented milk produced by the commercial yoghurt or mesophilic starter culture, LF221 and K7 strains did not have negative impact on organoleptic properties, while in cheese, produced from milk to which probiotics were added, a technological process had to be slightly modified. Survival was satisfactory in both carriers.

In conclusion, the evidence on *Lactobacillus gasseri* K7 and LF221 probiotic properties is accumulating and presents a good basis for the future studies on humans.

DNA sequencing and FT-IR spectroscopy as analytical tools to identify species of lactobacilli and bifidobacteria

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Precise identification of bacterial strains used in food processing is important for authenticity, safety and quality purposes. Members of the genera *Lactobacillus* and *Bifidobacterium* are the most used strains for probiotic purposes. Different molecular methods based on DNA, RNA and protein profile analysis are available for taxonomical classification, identification and monitoring of microorganisms.

Here we present a comparative analysis of partial *tuf* sequences for the identification and differentiation of two groups of lactobacilli: The *L. casei* group (5 species) and the *L. acidophilus* group (7 species).

A methodically different approach for the same purposes was made with FT-IR spectroscopy. Identification based on probability calculations is possible for the seven species of the *L. acidophilus* group.

The genus *Bifidobacterium* consists of more than 30 species and shows a great variety of physiological characteristics. However, the species' are genetically closely related and the 16S rRNA gene sequencing does not allow a clear distinction between some taxa. Therefore a method was developed based on the comparison of partial gene sequences encoding a phosphoketolase. The method is suitable for the phylogenetical study and identification of more than 31 species. As it is particularly capable of distinguishing between *B. animalis* and *B. lactis* and between *B. infantis* and *B. longum* it provides arguments in the current discussion about bifidobacterial taxonomy.

References

- Schleifer, K.H. and W. Ludwig, (1986), Phylogenetic relationships of lactic acid bacteria, p. 7-18. In Wood, B.J.B. and W.H. Holzappel (Eds.), The genera of lactic acid bacteria. Blackie Academic & Professional, Glasgow, UK.
- Chavagnat F., Hauelet M., Jimeno J., Casey M.G., (2002), Comparison of partial *tuf* gene sequences for the identification of lactobacilli. FEMS Microbiol. Lett. 217 (2), 177-183

The inhibitory effect of probiotic lactic acid bacteria towards pathogens

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Recently, a lot of attention has been paid to the health-promoting properties of certain acid bacterium strains, some of which have been promoted as probiotics. Examples are *Streptococcus* and *Bifidobacterium* that are utilized in yoghurt production and milk fermentation, or supplied as pharmaceutical preparations. Of particular importance is the potential of probiotics to reinforce mucosal defence, especially at the gastric and small bowel levels, for instance to protect against infections caused by *Helicobacter pylori* and *Salmonella enterica* serovar Typhimurium respectively. Possible underlying mechanisms of inhibition are the interspecies competition for nutrients, the prevention of pathogen adherence to epithelial cells, the production of antimicrobial substances by a resident or transient microflora, and/or an enhancement of the host immune response against pathogens. The PROPATH project of the EU PROEUHEALTH cluster aims to isolate and characterise the responsible antimicrobials to combat the Gram-negative pathogens mentioned above.

It is well known that probiotic lactic acid bacteria, in particular strains of species of the *Lactobacillus* and *L. casei* groups as well as *Bifidobacterium*, contribute to a stable gut microflora, in the prevention of the colonisation of pathogenic bacteria. This may be ascribed to the production of organic acids, resulting in a decrease of the local pH. Moreover, undissociated organic acids probably play a pivotal role in the anti-infectious activity of *Bifidobacterium* towards *E. coli* and *Salmonella*. On the other hand, several *Lactobacillus* strains produce antibacterial, acidic, low molecular mass, non-proteinaceous, heat-stable compounds with an inhibitory spectrum including Gram-positive and Gram-negative bacteria. For instance, *L. casei* Shirota, *L. johnsonii* La1, and *L. rhamnosus* GG produce antimicrobial compounds which have a synergistic effect with lactate and show strong antimicrobial activity against *S. enterica* ser. Typhimurium under acidic conditions.

Also, the widespread probiotic *L. johnsonii* La1 strain inhibits cell adhesion and cell invasion of enterovirulent bacteria both *in vitro* and *in vivo* (in mice), including both Gram-positive (e.g. *Staphylococcus aureus*) and Gram-negative (e.g. *Salmonella Typhimurium*) bacteria. However, the nature of the antimicrobial component(s) secreted by *L. johnsonii* La1 remains to be determined. Recent experiments indicate that supernatant of *L. johnsonii* La1, as well as an extract of it, inhibits the growth of *H. pylori* *in vitro*. This inhibitory activity was heat resistant, protease-sensitive, and independent of lactic acid.

Therefore, the overall inhibition of gastrointestinal pathogens by probiotics may be due to the synergistic action of organic acids, proteinaceous substances, and other hitherto unknown inhibitory agents.

Microbiological contamination of milk and milk products samples in 2002-2003

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At our Institute for food hygiene and bromatology on Veterinary faculty in Ljubljana, Slovenia we examine food of animal origin with microbiological and chemical analysis. Since 2002 we are accredited laboratory from the Netherlands and Slovenian accreditation council.

For analysis of milk products we use IDF standards. Our laboratory cooperates with inter-laboratory control FEPAS and our results are one of the top.

Milk samples and milk products samples are examined for the presence of *Salmonella* spp., *E. coli*, coagulase positive Staphylococci, *Listeria monocytogenes*, total count of microorganisms, *Clostridium* spp., *Yersinia enterocolitica*, *Proteus* spp., *Campylobacter jejuni*.

In the period between January 2002 and August 2003 we examined 441 samples of milk and 11193 samples of different milk products. According to law and regulations regarding microbiological demands for food in Slovenia 390 (88 %) milk samples and 1159 (97 %) milk product samples were appropriate. Among the inappropriate samples we have isolated either *E. coli* or the total count of microorganisms was too high.

Key words: microbiology, food hygiene, milk, milk products

Enterococci in dairy products: taxonomy, technological characteristics, safety and health promoting effects

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The history of the genus *Enterococcus* is strictly related to that of *Lactococcus* and *Streptococcus*. Nevertheless, according to the revised taxonomic outline of the Bergey's Manual of Systematic Bacteriology, they belong to different families, i.e. *Enterococcaceae* and *Streptococcaceae*, respectively, and the genera *Atopobacter*, *Melissococcus*, *Tetragenococcus* and *Vagococcus* seem to be the closest relatives to enterococci, as Gram positive bacteria with a low genomic G+C content.

The genus *Enterococcus*, as presently described, comprises 27 species, which can be grouped in several clusters according to phylogenetic analysis and phenotypic traits. Anyway the genus is heterogeneous, so that a reliable identification of environmental and clinical isolates is very difficult relying on traditional identification procedures.

Enterococci are associated with a number of fermented foods, such as cheeses. Strains belonging to the species *E. faecalis*, *E. faecium* and *E. durans* are the most frequently isolated in dairy products. They are also proposed as part of lactic starter culture for different European cheeses such as Fontina and Water-buffalo Mozzarella. The positive influence that enterococci may have on cheese is due to several specific characteristics: they may influence the flavour and taste of cheese with their metabolites, but can also produce several enzymes that interact with components of the milk. Some enterococcal strains of food origin are able to produce bacteriocins (enterocins) that are active against food-borne pathogens such as *Listeria* and *Clostridium perfringens* or towards spoilage bacteria e.g. *Propionibacterium* and *Clostridium tyrobutyricum*.

In addition, some *Enterococcus* strains have been studied and are used as probiotics, both in human prevention and in animal husbandry, to improve the microbial balance of the intestine and to reduce some infections. For example, *E. faecium* strain SF68, studied over a period of more than 20 years, has been shown to be effective in the treatment of intestinal disorders.

Despite these positive traits, attention should be paid to some safety aspects: a number of nosocomial infections, especially in immuno-compromised patients, have been reported as associated with enterococci, predominantly strains of *E. faecalis*, and to a lesser extent, *E. faecium*. This situation is further complicated by the presence, in some isolates, of transferable drug resistance genes that cause a more difficult control of the infections.

Food quality and safety, tracing in dairying

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All natural food is product of soil and/or water. There is the begin of food quality and safety regardless if it is vegetable or animal origin, being directly used as raw, transformed or processed by different methods in great variety of products. On its ways to consumer food can be harmful changed to lose its quality and become threat to consumer's health. Today, high levels of safety and safety are part of the major concerns in our European Society. Before food arrives to consumer are involved producers, processing plants and industries, sale organizations, different professions. Each of them is part of the chain, having defined tasks, activity and responsibility. Essential, to achieve proper quality, successful control, tracing and thus safety, is communication collaboration between links, especially those which are in interaction, without predominance over the others. Any link acting as self sufficient island system solution or ignorance of aut can turn a case into affair with harmful consequences.

The quality and safety in dairying starts on the farm with quality feed and water, feed handling and feeding the cows. Following is handling of cows in barns, milking parlours, during milking then milk handling. The problems can occur in any of these procedures and circumstances which have a cumulative effect and are the main sources of milk contamination. This can start from water, barn arrangement, environment, maintenance of milking systems, milking routine etc. slightly inconvenient environment or procedures can have harmful influence on the health of and an impact on milk production, its quality and safety. In cases of mastitis, which is cured by antibiotics, are at least ten common reasons that antibiotic contamination can occur in the milk such case the problems are in dairy industry and are threat to consumer's health. In 1992 WHO reported that through milk 28 infectious diseases is known which can cause diseases to humans. Dairying is so complex that only control and efficient tracing can eliminate harmful substances. Established must be integrative system for cooperation among agricultural, zootechnical, veterinary and human medicine services. This is priority task of authority responsible for food safety for dairy food traceability system has never been as present as in last few years. Food traceability an issue that needs tight cooperation of scientist and integration of knowledge from different New evolving IT systems enable development of automatic knowledge management systems are of great importance for prompt exchange of information's about activities and results.

In this respect the project "Food Quality and Safety in Dairying" is proposed to EU by a group of experts from AFEMA (working group for milk hygiene and mastitis prevention in Alpine, now exists).

Microwave milk testing

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During the milk receiving the most important quality parameters are: the fat content and the count of germ. Nowadays the examination is difficult and requires laboratorial background. We have been started our research with the aim of simplifying the milk testing procedure. Since many years our department is focused on researches in the field of microwave techniques, so we try to find a connection between the microwave properties and the quality parameters of milk.

First of all a cavity measuring circuit was developed, which is suitable for the examination of materials with low loss factor. The sample holder was placed into the cavity field. The frequency values were generated continuously from 2300 to 3200 MHz by a signal generator. We got our results based on the signs of the mW-meter and by the connection via measuring card to a PC.

The samples were fresh, raw milk with 3.6 % fat content, moreover consumptive milk with 2.8, 1.5 and 0.1 % fat content. The temperature of the tested milk was 20°C during the investigation. For germ count analysis we used fresh, raw, unpasteurized milk and milk with known high germ count originated from diseased cow.

We could determine the dielectric constant or permittivity (ϵ') with the knowledge of geometrical parameters of the sample holder and the measured frequency. During the measurement we seek for the minimum mW value and the belonging frequency (resonance- frequency). According to that we can calculate the dielectric constant.

During fat content measurements results, the continuous changement of the dielectric constant as the function of the fat content is clearly seen. The permittivity did not show any considerable differences through germ count examinations. However the dissipated output in the samples varies by the different germ counts.

Further investigation is needed for the clear understanding of the differences in dissipated output. By fat content measurements further milk samples needed with different fat content. Based on that, we will be able to draw a well determined function for the dielectric properties and the fat content.

Key words: cavity method, dielectric constant/ permittivity, resonance- frequency, milk fat content, germ count

Enumeration, isolation, and identification of bifidobacteria from dairy products

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Bifidobacteria are Gram-positive nonspore-forming irregular anaerobic rods. The typical habitat of this genus is human and animal intestinal tract. It has been suggested that *Bifidobacterium* species are important in maintaining general health. Potential health benefits have led to their wide application in dairy products. The aim of our study was to enumerate and isolate bifidobacteria from dairy products. The addition aim was to compare biochemical and PCR methods for identification of these isolates.

Six dairy products were tested. Bifidobacteria were enumerated and isolated using TPY agar modified by the addition of mupirocin (100 mg/l). Isolates were identified to the genus level by the detection of fructose-6-phosphate phosphotolase (F6PPK). Bifidobacteria were characterised using API 50 CHL and API ID 32 A Rapid tests. In addition, grow at 46 °C was tested. Subsequently, all strains were identified to the species level using computer program Bacter. The identification to the species level was also carried out by PCR method using species-specific primers. Amplified PCR product were analysed by 1 % agarose gel electrophoresis and visualized with ethidium bromide under UV light (wavelength, 260 nm).

The bifidobacterial counts in tested products varied from 2.37 to 7.17 log CFU/ml. High selectivity was seen for modified TPY agar from which all of the isolates were F6PPK positive and were identified as bifidobacteria. While most strains were identified as *B. animalis* using Bacter program, the same isolated had positive reaction with *B. lactis*-specific primers. Hence, it is not clear whether *B. lactis* and *B. animalis* are the same species, because Bacter database does not contain species *B. lactis* and on contrary, there are no available *B. animalis*-specific primers. On the whole, PCR primers could be genus-, species- or even strain-specific. Strain isolated from Rajio yogurt was identified as *B. longum* by Bacter as well as using PCR.

Our results showed, that most of the bifidobacterial strains currently used in food products could be of animal origin. One strain was identified as *B. longum*, but its bacterial counts in yogurt were too low. Further investigation should be focused on the selection of human bifidobacterial isolates, which are able to survive in milk for longer period of time. Also, the identity and origin of currently used strains should be cleared.

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Aflatoxins in food of animal origin

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Introduction

Reviewing international scientific and expert literature leads to the conclusion, that food safety is a priority in developed as well as in developing countries. By definition the term food safety means the prevention of risk for human health by permanent control of food during production, processing, storage, transport and final offer to consumers. In the case of food of animal origin, the risk is dependent to a great extent on the type and amount of contaminants in feed, consumed by production animals. In most cases contaminants are pathogenic microorganisms, especially salmonella, BSE, residues of veterinary drugs and other chemicals, and last but not least mycotoxins. Mycotoxins are toxic metabolites, produced by moulds under certain conditions. If they appear in food, human health can be threatened due to their carcinogenic, teratogenic, immunosuppressive and other effects. Most important mycotoxins are aflatoxins, ochratoxins, zearalenone, fumonisins as well as deoxinivalenol, T-2 toxin and other trichothecenes. Among them, aflatoxins are most frequent and most important due to high toxicity, particularly aflatoxin B₁ and its metabolite in milk aflatoxin M₁. Aflatoxins are the only mycotoxins, whose concentration in feed for production animals and food of plant and animal origin is regulated by law in Slovenia and worldwide.

Method of analysis

A method of analysis with the limit of detection and limit of quantification appropriate for analysis of aflatoxins in feed and food was introduced in our laboratory. It consists of sample clean-up using immunoaffinity columns and liquid chromatography with postcolumn derivatisation and fluorescence detection. The procedure was validated according to requirements of Commission Decision 2002/657/EC, Commission Directive 98/53/EC and Commission Regulation No. 466/2001/EC. Obtained validation parameters demonstrated that the procedure is suitable for determination of aflatoxins at the concentration levels as regulated.

Results and discussion

In the last three years, 291 samples of food of animal origin (mainly eggs and liver) were analysed on aflatoxin B₁ and 163 samples of milk and dairy products were analysed on aflatoxin M₁. Most samples were collected within regular inspection for undesirable substances in food. The concentration of aflatoxin B₁ and M₁ was below the detection capabilities of above mentioned detection procedures. With respect to data provided by the FAO for the year 2002, eggs, pig liver, and pork contained maximally 0.4 ppb, 0.5 ppb, and 1.04 ppb of aflatoxin B₁, respectively, and cow milk maximally 0.33 ppb of aflatoxin M₁. Therefore, we can conclude that food of animal origin do not present serious risk for human health in Slovenia. The same indicate our data on aflatoxins content in feeds. In the last three years, none of 311 analysed feed samples exceeded maximum permitted concentration levels of aflatoxins. Despite the relatively small part of aflatoxins consumed by feed is being deposited into meat, internal organs, eggs and milk, the warning has to be raised as opening of the market and free circulation of feed stuffs after joining the EU poses serious risks for human health if not tightly controlled and regulated.

Comparative examining of antibiotic residues in milk by enzyme, receptor-enzyme and inhibitive procedure

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Milk is used for consumption and it must be health accurate. One of the parameter in accuracy is proving presence of veterinary drug residues. The main aim of the work was to compare methods for determining presence of antibiotics in milk with the purpose to choose the most method that could prove quantity that is maximally permitted by the Codex Alimentarius Commission.

Enzyme method (Penzym S test), receptor-enzyme method (Delvo-X-press (-lactam II) and biological methods (Delvo SP test, diffusion method with wholes in agar and B. stearothermophil microorganism) were examined. For determining sensitivity of the method in milk without antibiotic were added different concentrations of standard drugs. Three methods were chosen and the sample milk were compared: 51 samples from different owners that are in ransom place, 20 sample tank on the farm, 10 samples from ransom place, 68 samples from different parts of transport 29 samples from tanker truck milk, 20 samples from pasteurized milk from the market.

The threshold for detecting all four methods according to penicillin G, ampicillin and an was within the frame of MRL. The detection threshold according to cloxacilline determined by Diffusion method and Delvo-X-press was above MRL value of 5 µg/kg, while detection threshold determined for Penzym S test and Delvo SP test was within the frame of maximally permitted quantity of cloxacilline in milk. Kappa statistical analyses was used for comparing the results obtained by Delvo SP test, Penzym S test and Diffusion test in parallel examination of milk samples taken different spots in a production chain. Mutual accordance for all the tests for the investigated samples was discovered and it was in the interval of kappa values not lower than 0.61-0.80, interval of almost ideal coincidence).

High percentages of milk samples that contain antibiotic residues impose a need for regular milk examination by applying appropriate screening methods. We suggest the samples examined by Delvo SP test in the line of ransom. If the presence of antibiotics residues is proven necessary to examine positive milk samples on line using Penzym S test or Delvo-X-press test of high probability that residues of beta lactam antibiotics are present and these two tests can in the further examining in the dairy, one of these two tests may be used, because it is necessary great number of the samples, and both tests are faster than Delvo SP test. By recurrent examination, starting with the milk from cistern, milk from ransom station or milk from certain owner specific milk that was contaminated by antibiotics can be traced. By analogous recurrent procedure can also be traced what is the cistern that contained milk that contaminated the whole line.

Detecting the residues of veterinary drugs in raw milk could provide supply of good milk influence the consumers and could reduce harmful consequences that appear in milk processing. Applying penalty measures could contribute towards developing responsibility of milk processor when prescribing therapy and selling milk, and this would reduce presence of veterinary drug residues in milk.

Key words: milk, antibiotics, MRL

Microbiological quality of some critical control points in the cheese production of individual Slovenian cheesemakers

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The microbiological quality of 98 samples taken at some critical control points during milking and processing of 14 semi-hard cheeses made from raw cow's milk by individual Slovenian producers was studied. The sampling points were: swabs from milking machines before and after milking, fresh raw and mixed milk from a vat, whey immediately after curdling, salt water, cheeses after one month of ripening and after one month of being kept vacuum packed at 6 °C. The total number of micro-organisms on 1 cm² of milking machines surfaces before and after milking was $14.7 \cdot 10^3$ and $15.0 \cdot 10^3$ cfu, respectively. The number of non-lactic-acid producers was 3 times higher than lactic-acid producers, while the number of coliforms and enterococci was 230 and 120 cfu, respectively. The high number of micro-organisms on the surfaces of washed milking machines before milking showed ineffective cleaning (washing) by about 60 % of cheese producers. There were no seasonal differences in the number of micro-organisms, except that the number of coliforms was higher in spring. The total number of micro-organisms was $4.0 \cdot 10^5$ cfu/ml in raw milk and $6.0 \cdot 10^6$ cfu/ml in mixed milk from a vat (raw fresh milk and milk incubated for about 18-24 hours at room temperature) and it did not grow significantly during cheese processing. The lactic-acid and non-lactic acid producers in both types of samples were at a ratio of 1 to 1. The number of coliforms in raw and mixed milk was in the range $3.5 \cdot 10^5$ and fell to $5 \cdot 10^4$ in whey. The average number of enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactobacilli, lactococci, proteolytic and lipolytic micro-organisms in milk and in whey were in the same logarithmic range of about $2.0 \cdot 10^4$, 300, $3.3 \cdot 10^4$, $1.0 \cdot 10^6$, $6.0 \cdot 10^3$ and $1.6 \cdot 10^4$ cfu/ml of the sample, respectively. After one month of ripening the average total bacterial count was $9.2 \cdot 10^7$ cfu/g of cheese, of these $6.8 \cdot 10^7$ represented lactic-acid producers and $2.2 \cdot 10^7$ represented non-lactic acid producers. The average number of coliforms, enterococci, aerobic spore-forming micro-organisms, yeasts and moulds, lactococci, lactobacilli, proteolytic and lipolytic micro-organisms were $2.0 \cdot 10^5$, $6.3 \cdot 10^6$, 280, 960, $2.5 \cdot 10^7$, $9.8 \cdot 10^7$, 450 and $9.8 \cdot 10^4$ cfu/g of cheese, respectively. After two months of keeping vacuum-packed cheeses at 6 °C, the number of micro-organisms did not rise significantly, except for the number of yeasts and moulds which grew to $3.6 \cdot 10^4$ cfu/g of cheese. The total number of micro-organisms in salt water was about $4.0 \cdot 10^6$ cfu/ml, while a high number of enterococci ($5.0 \cdot 10^4$ cfu/ml), yeasts and moulds ($6.0 \cdot 10^4$ cfu/ml), lactococci (10^8 cfu/ml) and lactobacilli ($2.0 \cdot 10^7$ cfu/ml) was also established. There were no significant differences in number of micro-organisms between spring and autumn season, except for enterococci ($P=0.0004^{***}$) and lipolytic micro-organisms ($P=0.0002^{**}$). Significant differences between the microbiological quality of samples from individual cheese makers were also established ($P<0.0001^{***}$). There is no statistical significant influence on microbiological quality between different areas, with or without starter cultures added at the beginning of cheese production, or feeding (pasture, feeding in a cowshed). It is suggested that more importance should be given to milking and cheese production hygiene, as well as the determination and control of critical control points in firms for improved cheese quality and the prevention of food-borne pathogenic outbreaks.

Residues of environmental pollutants and some veterinary drugs in raw milk

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From people health preservation and improvement's point of view and with the intention to assure hygienic suitability of food of animal origin, we investigated and presented the level of pollution of raw milk with organochlorine and organophosphorus pesticides, polychlorinated biphenyls, Pb and Cd, antibiotics, chloramphenicol, sulphonamides and ivermectin on restricted Štajerska region which is a part of Slovenia. We separated it into four groups depending on the influence of the industrial milk production, intensive, extensive. We analyzed the samples, which were acquired ourselves separately in two time periods, in year 1999 and 2002.

In the year 1999, there were 30 samples of raw milk taken in the spring, summer, and autumn, which were then analyzed for the residues of organochlorine and organophosphorus pesticides, polychlorinated biphenyls (samples from the winter were not analyzed), trace elements as mentioned above and antibiotics, chloramphenicol, sulphonamides and ivermectin residues. In all analyzed milk samples there were only low contents of majority of environmental pollutants found. Slightly higher contents were found only with the total DDT, especially with the samples from more polluted sources, which the diminished during our investigation. Based on the content of total DDT we confirmed the influence of industry and major use of pesticides on this type of pollution and also the influence of the intensive rural and milk production. The polychlorinated biphenyls did not present a problem on the restricted Štajerska region discussed, because in all the samples from in the seasons of the year there were only very low contents determined. Contents of the trace elements (Pb and Cd) in the analyzed samples did not present a problem, because they were all below the detection limit of the method we used for analyzing. Antibiotics, sulphonamides and ivermectin were not found in any analyzed raw milk samples, acquired in autumn 2002 we confirmed the presence of chloramphenicol in concentration $<0.2 \mu\text{g/kg}$ and $0.5 \mu\text{g/kg}$. The results of milk from Štajerska region were then compared to the results of systemic veterinary-sanitary inspection from years 2000 and 2001. Based on the results of analysis of raw milk contents within systematic veterinary-sanitary inspection from years 2000 and 2001 we determined, that milk from the entire Slovenia did not present a problem. The contents of organochlorine pesticides and trace elements were under the detection limit in all the analyzed samples. We also didn't detected any antibiotics, chloramphenicol, sulphonamides and ivermectin residues.

The comparison of milk pollution with total DDT from the discussed Štajerska region and entire Slovenia showed a slightly higher level of pollution from discussed region.

HOLDBAC Listeria a protective culture to destroy Listeria sp. in food

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Protective cultures are healthy and safe microorganisms, which are added to foods in order to inhibit poisoning and other undesired microorganisms. HOLDBAC Listeria is a *Lb. plantarum*, which was isolated in 1992 in France. It is considered as GRAS and patented. HOLDBAC Listeria acts highly specific against listeria by forming a Pediocin AcH. Consisting of 44 amino acids this pediocin acts by making the cell membranes of target organisms permeable. It acts very specific especially against listeria monocytogenes-types, is heat stable and resistances against it are not known at the moment. The pediocin is deactivated very quickly in the intestines by peptidases. HOLDBAC Listeria is being used already successfully in many segments of the food industry (especially cheese and meat) where listeriae are responsible as spoiling contaminants. As diseases caused by listeriae are gradually increasing HOLDBAC Listeria might be an interesting alternative to increase food safety by natural means.

Inhibition of *Escherichia coli* O8K88H9 ent by *Lactobacillus gasseri* K7 - in vivo study gnotobiotic piglets

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The adhesion of *Lactobacillus gasseri* K7 to intestinal mucosa and its inhibitory activity *Escherichia coli* O8K88H9 ent⁺ was studied *in vivo* on gnotobiotic piglets. In addition we tried to find that probiotic K7 strain is safe for application. The three groups of gnotobiotic piglets were treated with *E. coli*, K7 or both bacteria. Competitive exclusion of *E. coli* by K7 strain was studied by comparing the viable counts of *E. coli* and K7 strain, in contents and on mucosa of duodenum, jejunum, ileum and colon. Qualitative analyses of organic acids production were conducted by using pH value of intestinal contents. Quantitative analyses of organic acids in intestinal contents were performed by capillary isotachopheresis. The haematological analyses, comprising determination of number of leucocytes, share of lymphocytes, concentration of total immunoglobulins, and index of phagocytic activity, metabolic activity of phagocytes and proliferation of lymphocytes were performed to establish the stimulation of the immune response. The iodo-nitro-tetrazolium reductase test (LACHEMA, Czech Republic) was used for measuring metabolic activity of phagocytes. Proliferation of lymphocytes was measured by the colorimetric Cell Proliferation ELISA, BrdU (Roche, Germany). Other parameters were determined by common haematological analyses. Proteomic analysis gave us insight into infection intensity in gnotobiotic piglets, inoculated by *E. coli*. The safety of K7 strain was studied by clinical observations of animals and morphological analyses of intestines, using electron microscope. The results of our study showed that *Lactobacillus gasseri* K7 survived and temporarily colonised the intestine of piglets. Different mechanisms, such as competitive exclusion, production of organic acids and stimulation of immune response, were involved in the inhibition of *E. coli* by K7 strain. Clinical observations of experimental animals showed the positive effect of *Lactobacillus gasseri* K7 on health and condition of piglets. Additionally, we did not observe inflammatory changes in intestines of piglets treated with K7, which confirmed its safe use. The results of our study represent a contribution to better knowledge of mechanisms of establishment and regulation of intestinal microflora by oral application of probiotic bacteria. Proved functional effects of K7 strain *in vivo* and confirmation of its safety are prerequisites before probiotic strain can be used in human clinical studies.

Application of interspecific protoplast fusion for construction new hybrid probiotic bacteria inhibited some of mycotoxigenic Fungi growth in yoghurt

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The technique of protoplast fusion is potentially an important tool for the genetic manipulation of lactic acid bacteria and hence strains improvement.

Attempts were made by inter-specific protoplast fusion between *Lactobacillus casei* subsp. *rhamnosus* and *Lactobacillus reuteri* to construct new fusant that could grow in the presence of mycotoxigenic fungi in specific media and yoghurt.

Decontamination of some mycotoxigenic fungi such as *Aspergillus parasiticus*, *Aspergillus ochraceus* and *Fusarium proliferatum* with parental and fusant strains in specific media and yoghurt appeared that parental and some fusant strains inhibited growth of mycotoxigenic Fungi to some degree.

Moreover, detoxification of aflatoxins with parental and some fusants appeared that *Lactobacillus casei* subsp. *rhamnosus* and some fusants have removed aflatoxin B1 from solution.

"Trentingrana" cheese: sensory profiling and PTR-MS analysis of volatiles emissions

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We describe the preliminary investigation of the sensory quality of "Trentingrana" cheese, a variety of Grana Padano, the well known Italian hard cheese with PDO (Protected Designation of Origin), which is produced only in a limited mountainous region in northern Italy (Trentino) with its own trademark "Trentingrana". The aim of this work is the study of the distinctive sensory features of this cheese bringing together different institutions and know-how: sensory scientists, statisticians and physicists.

Quantitative descriptive analysis was applied by a panel of trained judges using a developed vocabulary which contains 30 sensorial parameters of physical aspects, texture, odour, taste, and aroma. In all, 71 samples have been evaluated: 41 of Trentingrana, coming from the main factories located in Trentino area at different ripening degree (from 8 to 31 months), 15 of Grana Padano (from 18 to 24 months), and 15 of Parmigiano Reggiano (from 24 to 28 months), from the most representative factories located in their respective production areas.

We investigate the relevance of each sensory attribute on the typical characteristics of this cheese and estimate, in terms of perceived quality, the degree of homogeneity of the products. For Trentingrana we also followed the changes in the sensory profiles during ripening.

Peculiarities and similarities of this cheese in comparison with the more famous competitors are discussed.

A second goal is to compare the odour/aroma sensory profile, obtained by the panel with the head-space analysis performed by means of PTR-MS (Proton-Transfer-Reaction Mass-Spectrometry). This technique has been proposed for several applications, from environmental to medical fields, and it is getting more and more used and recognised as an important, fast, simple and sensitive tool for Volatile Organic Compounds (VOCs) detection.

We analysed by direct PTRMS analysis of the headspace of just grated cheese held at 36 °C without any preparation or concentration and in parallel by the sensory panel 20 Trentingrana cheeses, produced in 10 factories belonging to the Consortium with two different ripening degrees.

The data indicate that this instrumental analysis is a useful tool for detecting changes in odour profile during ripening. Multivariate statistical data analysis shows that the method allows sample discrimination comparable with the sensory profiling. The PTRMS spectra represent not only a simple and rapid fingerprint of samples useful for classification/discrimination but chemical information can be derived from the mass peaks allowing the identification and quantification of compounds responsible for the differences among the samples.

Enterococci from feed - potential probiotics

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Microbes from many different genera are being used as probiotics. The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; lactobacilli, enterococci and bifidobacteria. Nowadays, probiotics are mostly used in food-dairy industry. However, feed industry is also open for using of this living microorganisms which upon ingestion in certain numbers exert health benefits into host organism. Our study includes selected enterococci from canine commercial feed which were genotyped by tDNA-PCR followed by capillary electrophoresis and tested for their ability to adhere to intestinal mucus, to produce antimicrobial compounds, to survive in the presence of salt bile and for their sensitivity or resistance to antibiotics. Moreover, production of lactic acid and their urease activity were also tested. All testing strains were performed to follow one direction i.e. selection of suitable further probiotic strain for their application as feed additive. The strains were allotted to the species *Enterococcus faecium*, *Ent. faecalis* and one strain were detected as *Ent. hirae*. The strains tested were well adhesive to human as well as to canine mucus. Even they adhere better to human mucus than to canine mucus. All strains were kanamycin and gentamycin resistant and 3 of them were also resistant to erythromycin. Lactic acid production was found in the range from 0.8 up to 1.3 mmol/l. Urease activity of those strains differed from low to high levels in nkat/ml. Bacteriocin production was not found against target of closed related strains. They were able to survive in the presence of 5 % oxgall (bile salt). It must be interlinked that especially high adhesive abilities of the tested strains represent good conditions to develop new probiotics among them. In addition, adhesion ability of enterococci to different mucus is presented as new phenomenon. Moreover, host specificity in this condition (adhesion) was confirmed by us. It is in contrary to lactobacilli in which adhesion is predominantly associated with host organism.

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Enterococci in cheeses - phenotypization and antibiotic resistance

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The material of investigation was consisted of samples of fresh and ripened cheeses made from raw and cooked milk subjected to rennet or acid coagulation. The primary isolation of enterococci was carried out on kanamycin-aesculine-azide agar at 37 °C and 42 °C during 24 hours. It was isolated totally 42 strains of enterococci. The examination of antibiotic resistance/sensitivity profile was performed by applying disk-diffusions procedure on Muller-Hinton agar. The number of enterococci determined in cheese samples depended on applied technological process. In the samples obtained by rennet coagulated cheeses made from raw and cooked milk the number of enterococci ranged from 8.0×10^4 to 9.0×10^6 cfu/g. and 4.0×10^7 - 4.4×10^7 cfu/g, respectively. In the case of ripened cheeses made from raw and cooked milk subjected to acid coagulation the number of enterococci was ranged from $<10^2$ to 5.0×10^4 cfu/g and 2.5×10^4 - 1.5×10^5 cfu/g, respectively. In the case of samples of fresh cheeses made from raw and cooked milk subjected to acid coagulation the number of enterococci was ranged from 2.4×10^5 - 2.12×10^7 cfu/g. and 5×10^4 - 6×10^4 cfu/g, respectively.

The phenotypic identification of isolated enterococcal strains was performed according to following biochemical-physiological characteristics: microscopic examination (cell morphology), catalase activity, growth in MRS broth at 10 °C and 45 °C, growth at pH 9.6, growth in broth containing 6.5 % NaCl, growth in 0.1 % methylene blue milk, resistance at 60 °C/ for 15 and 30 minute, Voges/Proskauer reaction, fermentation of ribose, reduction of 2,3,5 - triphenyltetrazolium chloride (TTC), pigmentation on Tryptone Soya Agar and utilization of raffinose and mannitol. The isolate strains of enterococci were resistant to following antibiotics: penicillin (65.82 %), tetracycline (62.02 %), lincomycin (68.35 %), gentamycin (27.84 %), neomycin (31.64 %), erythromycin (31.64 %) and chloramphenicol (65.82 %).

Key words: enterococci, cheese, phenotypization, antibiotic resistance

Immunomodulatory effect of *Lactobacillus rhamnosus* GG from low fat fresh cheese "BioAktiv LGG"

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Various immune responses have been influenced by probiotics and these immunomodulatory effects have been proposed for several potential applications such as the prevention of infectious diarrhoea, alleviation of hypersensitivity reactions and tumour suppression. The new probiotic product from LURA d.d., low fat fresh cheese "BioAktiv LGG", contains well-known probiotic strain *Lactobacillus rhamnosus* GG (LGG). The viable count of *L. rhamnosus* GG remained stable and has been 1.3×10^8 CFU/g of low fat fresh cheese "BioAktiv LGG" during storage of 16 days at 4 °C. The effect of *L. rhamnosus* GG from this product on the immune response, and its survival/residence time in the gastrointestinal tract in Swiss albino mice was also studied, because this is an important point in relation to immunomodulation. During the feeding of mice with *L. rhamnosus* GG, with a daily dose of 2.2×10^{10} cells, the number of lactobacilli in faeces was increased and reduction of enterobacteria and sulphite-reducing clostridia was observed. The similar results were obtained in homogenates of large intestine of mice in the 1st and in the 14th day after feeding with *L. rhamnosus* GG, which pointed out the adherence ability of examined strain. Furthermore, the oral immunization of Swiss albino mice was performed with a dose of 2.2×10^{10} *L. rhamnosus* GG cells per day, for eight consecutive days. The blood samples were collected on the 4th, 8th, 10th, 14th, 17th and 21st day after the 1st immunization. The sera of mice were tested for total and specific anti-lactobacilli IgA, IgG and IgM antibodies by ELISA assay. *L. rhamnosus* GG slightly stimulated the total humoral immune response but didn't evoke reaction against itself, which confirms its probiotic properties and make new probiotic product, low fat fresh cheese "BioAktiv LGG", as good carrier for this probiotic strain.

Characterization of *Lactobacillus plantarum* strains as probiotics

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Probiotics are live-bacteria containing foods, which, upon digestion, exert beneficial effects to host. The most common bacteria intended for probiotic use are lactic acid bacteria and Bifidobacteria, normal inhabitants of gut microflora. *Lactobacillus plantarum* is an important species in the fermentation of various plant products, and it is known to produce antimicrobial substances that are active against certain pathogens.

There are several requirements for a bacterium to be selected as probiotic. Of these we studied tolerance to acid and bile salts, ability to ferment fructooligosaccharides, beta-galactosidase activity and susceptibility to antibiotics for a total of 15 strains including 13 strains of *Lactobacillus plantarum*, 1 strain of *Lactobacillus acidophilus* and 1 strain of *Lactobacillus pentosus*.

Acid and bile tolerant isolates were *L. plantarum* 80, *L. plantarum* DSM 20174, *L. plantarum* DSM 20246, *L. plantarum* 37, *L. plantarum* HU, and *L. acidophilus* ATCC 4356. 8 strains had the ability to ferment fructooligosaccharides. These were *L. plantarum* 80, *L. plantarum* DSM 20174, *L. plantarum* Strains *L. plantarum* 80, and *L. plantarum* NCIMB 1193, *L. plantarum* 37, *L. plantarum* 73, and *L. plantarum* NCIMB 1193, and *L. pentosus* had the highest beta-galactosidase activities.

According to these results, *L. plantarum* 37, *L. plantarum* 80, *L. plantarum* NCIMB 1193, and *L. plantarum* HU were the most successful strains in the study.

Key words: Probiotic, *Lactobacillus plantarum*, Acid and Bile Tolerance, Beta-galactosidase, Fructooligosaccharide, Antibiotic Susceptibility.

Evaluation of rheological methods for the characterisation of yogurt and cheese

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The texture profiling of yogurt

The objective of this study was to measure the rheological characteristics from a "soft solid" product like a yogurt „as it is“, without partial or complete destruction of the weak structure. A key problem in measuring soft foods by rheological techniques is that placing the sample into the rheometer between a parallel-plate or a cone-and-plate measuring system partially destroys the structure we want to be measured. The answer of the problem is the vane geometry. When the vane is inserted into the sample, there is only slight damage close to the blades. Another advantage of the vane geometry is the elimination of serious wall-slip effects. For most purposes in the dairy food industry, comparative measurements are sufficient and absolute data are not always necessary.

We have evaluated two different methods using a rheometer from Physica (MCR 300) equipped with the vane geometry and compared the data with the uniaxial-compression test. The measurements are easily accomplished and fast. A good correlation was obtained between the uniaxial-compression test and a controlled shear stress experiment (rotation) and an amplitude sweep (oscillation). Results and data of different yogurts are shown and discussed.

The same rheometer allowed us to measure the:

Melting quality of Swiss Raclette cheese

The melting properties of Raclette cheese are very important for the cheese manufacturer and the consumer. The aim of this study was to measure the "melting point" of Raclette cheese by rheological methods. A thin disk of cheese was measured between a parallel-plate measuring systems (Rheometer Physica MCR 300) under constant oscillatory conditions. The following parameters were selected: deformation 0.5 %, angular frequency 10/s. The parallel-plate measuring system consisted of two moulded plates. The temperature was raised from 20 °C up to 80 °C in about 30 minutes and the storage- (G') and loss- (G'') module were observed. The crossover of G' and G'' is called "melting point" and correlated with the softening point and the dropping point, determined by another instrument (Mettler FP 80 & 83). The value of the "melting point", determined with the rheometer, lies between the softening point and the dropping point. We discovered a good correlation between the maximum of the loss angle (tan δ) and the softening point as well as the dropping point. This temperature sweep technique allowed us to discriminate between Raclette cheese of better and poorer melting qualities and between Raclette cheese and processed cheese.

Results and data of 35 different Raclette cheeses are shown and discussed.

Identification of seven species of the Lactobacillus acidophilus group by FT-IR spectroscopy

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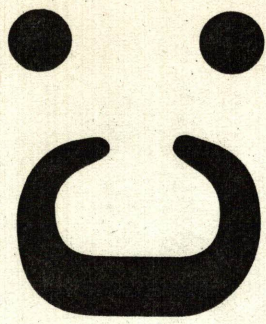
Precise identification of bacterial strains used in food processing is important for authentication and quality purposes. The boom of recent genomic analysis leads to a new knowledge phylogenetic relationship of species and to taxonomic groupings of related species, i.e. the definition of an "Acidophilus group" including 7 species of lactobacilli (Schleifer, K.H. and W. Ludwig, 1999). The institute established a method of lactobacilli species identification by partial gene sequencing and proposed numerical similarity calculations of type strain relationship (Chavagnat et al., 2002).

In order to improve the identification accuracy we searched for an additional method, independent of molecular biology techniques. The identification of microorganisms by vibrational spectroscopic techniques has found increasing interest and is applicable as a rapid and reliable method mainly in biomedicine (Maquelin et al., 2002) but also in the characterisation of non-genetic bacteria such as lactic acid bacteria (Amiel et al., 2000; Weinrichter et al., 2001).

The success of Fourier Transform Infrared (FT-IR) spectroscopy for this purpose is only possible with efficient chemometric algorithms and powerful microcomputers. In our work we collected FT-IR transmission spectra of 74 strains of the seven species of the *Acidophilus* group mentioned above. Our dataset now contains 376 spectra. Outliers have been detected by visual inspection and by PCA analysis of group of spectra and were eliminated. Various procedures of pre-processing and classification have been tested and compared with respect to their predictive ability. The most encouraging results were achieved by linear discriminant analysis (LDA) of the absorbance values at selected wavenumbers of the normalized spectra. The percentage of correct species assignment in cross-validation procedure with one spectrum left out for model building) were 95, 95, 69, 100, 98, 100, and 91 % for *L. acidophilus*, *L. amylovorans*, *L. crispatus*, *L. gallinarum*, *L. gasseri*, *L. helveticus*, and *L. johnsonii* respectively. Very distinct grouping was found for *L. gallinarum* and *L. helveticus*, the most difficult differentiation LDA was between the pairs *L. crispatus* / *L. amylovorans* and *L. gasseri* / *L. johnsonii*. Alternative approaches such as common spectral searching algorithms (e.g. Pearson correlation, Euclidean distance, Artificial Neural Networks) will be evaluated in order to find the most reliable, robust and practical method of spectral pre-processing, selection of spectral windows and identification algorithm(s) to use for 'routine' FT-IR spectroscopic identification of the species of the *Acidophilus* group.

References

- Schleifer, K.H. and W. Ludwig, (1995), Phylogenetic relationships of lactic acid bacteria, p. 7-18. In Wood, B.J.B. and W.H. (Eds.), The genera of lactic acid bacteria. Blackie Academic & Professional, Glasgow, UK.
- Chavagnat F., Haueter M., Jimeno J., Casey M.G., (2002), Comparison of partial *tu* gene sequences for the identification of lactobacilli. *FEMS Microbiol. Lett.* 217 (2), 177-183
- Amiel C., Marley L., Cork-Dauble M.C., Pichon P., Travers J., (2000), Potential of Fourier Transform Infrared Spectroscopy for discrimination and identification of dairy Lactococcus bacteria. *Lett.* 80, 445-459
- Maquelin K., Kirschner C., Choo-Smith L.P., van den Braak N., Endtz H.P., Naumann D., Puppels G.J., (2002), Identification of lactic acid bacteria by vibrational spectroscopy. *J. Microbiol. Methods*, 51, 255-271
- Weinrichter B., Luginbühl W., Rohm H., Jimeno J., (2001), Differentiation of facultatively heterofermentative lactobacilli from milk, and hard type cheeses by SDS-PAGE, RAPD, FTIR, energy source utilization and autolysis type. *Lebensm.-Wiss. Technol.* 34, 100-108



Origin and Authenticity of Dairy Products

Growth of probiotic bacteria in reconstituted whey

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Whey is nutritive very valuable byproduct in the cheese industry, which is unfortunately still not used enough in food industry. The whey contains proteins of the highest biological value, affluent mineral content and B group of vitamins. Considering nutritive value and health benefits of whey, the purpose of this research was to explore the possibilities of using whey as a fermented drink. The first step was to determine the growth of probiotic strains *Lactobacillus acidophilus* La-5, *Bifidobacterium bifidum* Bb-12 and *Lactobacillus casei* Lc-01 in reconstituted sweet whey with 6 % of total solids at 37 °C. The effect of prebiotic inulin addition on fermentation and bacteria survival during 28 days of storage at approximately 5 °C was also investigated. Low heat pasteurization, at 63 °C/30 minutes, showed unsatisfactory results therefore for further research pasteurization at 74 °C / instantly was applied. Generally, increase of viable cells of all investigated strains of bacteria during 24 hours of fermentation was small, in average one logarithmic unit. During fermentation, inulin addition had no effect on bacterial growth and on sensory properties of fermented whey. Sensory scores of all fermented beverages were the best after 18 hours of fermentation. The lowest sensory score had whey fermented with *Bifidobacterium bifidum* Bb-12 strain because unacceptable flavour and taste. Whey fermented with *Lactobacillus acidophilus* La-5 or *Lactobacillus casei* Lc-01 strain showed satisfactory sensory characteristic.

Key words: whey, fermentation, probiotic bacteria

Adhesion capability of lactobacilli to human and animal intestinal mucus

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The benefits of using lactic acid bacteria in the food and also in feed chain, both through consumption are increasingly recognized by the food/feed industry and consumers alike. The tory environment surrounding these products is diverse, covering e.g. foods, food ingredients additives or dietary supplements. Microorganisms which are promising for the use indicated; include several important abilities that are source (origin) of it, anti-microbial effect, surviving different pH, acid and bile tolerance and the important phenomenon-adhesion ability. Therefore tobacilli which were selected from different sources such as canine faeces and feed, ileum of en, calf's intestine or vegetable salad were tested for their ability to adhere to human and ani (canine) mucus. Adhesion ability of the strains tested to human mucus ranged from 1.5 to 8.3 *Lactobacillus* sp. AD2 from canine faeces showed the highest activity (8.3 %). On the other he *Lactobacillus johnsonii* 4982 from vegetable salad had the lowest adhesion ability to human r Adhesion to canine mucus ranged from 1.6 (*Lbc. johnsonii* 4982 - vegetable salad) to 13.0 % (*Lactobacillus* sp. LK1 - vegetable salad). Generally, ability of strains to adhere to both mucus well balanced. Even, the highest adhesion to canine mucus was detected by the isolate from eable salad. Isolates from canine feed were well balanced in their adhesion to both mucus (h 4.2-5.1 %; canine-3.1-4.0 %) as well as isolate from canine faeces (human-2.7-8.3 %; canine-2 %). Strain AD2 from canine faeces showed well-balanced adhesion to both mucus (8.3; 6.0 %) higher to human than to canine mucus. It was also applied to dogs with different disorders of digestive system and its benefit was found (e.g. decrease of cholesterol, anti-diarrhea influen was selected for the other probiotic testing.

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Weakly structured fluids: a recurrent item in the present rheological research

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Several food products as well as many industrial fluids display non-Newtonian character with marked changes in viscosity and other material functions. At low strains and stresses the rheological behavior is governed not only by the molecular and/or microscopic features of dissolved and/or disperse species, by also, and even more, by the higher-order structures resulting from mutual interactions between these components. Even when they impart high viscosity and marked elastic properties in the linear regime, such structures are frequently weak and fragile, so that they undergo a substantial breakdown when the applied stresses exceed a narrow critical range. Several systems belonging to different industrial sectors can be grouped together and classified as weakly structured fluids, in virtue of their similar rheological properties in both linear and nonlinear regimes.

In several aqueous biopolymer systems the supermolecular structures and rheological properties differ from those of chemically crosslinked polymers, where covalent bonds exist between the polymer chains, and from those of concentrated polymer solutions and melts, where temporary networks are formed by topological interchain interactions. Coulombic, van der Waals, dipole-dipole, hydrophobic interactions and hydrogen bonds often act cooperatively to form quasicrystalline junction zones between two or more macromolecules, so that, at sufficiently high polymer concentration, chain-chain associations can give origin to gel microdomains or span the whole system to form a three-dimensional network. Weak gel is the term usually adopted to distinguish their linear viscoelastic and flow properties from those of other neighbouring polymeric systems. Similarly, extended networks exhibiting weak gel behaviour are frequently formed by colloidal particles through different aggregation mechanisms due to prevailing short-range attractive interparticle forces or to the action of adsorbed and non-adsorbed components (bridging and depletion flocculation). As in the case of polymer chain associations, interparticle bonds are more or less sticky depending on the balance between attraction and thermal energy and, correspondingly, aggregation structures can display reversible behavior or impart irreversible characteristics to the disperse system.

Starting from '80s an increasing scientific and technological interest has been focussed on the rheological properties of weakly structured fluids, being developed on both experimental and modeling sides, in virtue of more sophisticated instruments and simulation techniques. The present contribution is intended as an excursus through the different aspects of the rheological research with the aim of underlining the peculiar features of such systems.

New approaches in studying microbial diversity of dairy products

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The quality and sensorial properties of dairy foods are strongly associated with specific attributes of raw milk, with the climate and environmental conditions, with the cheesemaking technologies and with the microbiota responsible for the fermentation process and ripening. Biodiversity of these microorganisms is considered valuable for the quality of dairy products. The study of biodiversity has been considerably improved by application of nucleic acid based methods, efficient tools in addressing new questions concerning the complex microbial communities of dairy products.

The first approaches to the DNA-based analysis of microbial population were the study of restriction fragment pattern, such as RFLP and PFGE. The development of a number of PCR techniques, such as RAPD, ERIC, REP-PCR and AP-PCR increased the sensitivity of the molecular methods, being able to discriminate at strain level. The application of these techniques allowed the monitoring of population dynamics during fermentation and ripening of dairy products. Since the biodiversity studies require efficient identification of microbial species, different methods based on the analysis of genes coding for ribosomal RNA have been developed and applied. Most of the molecular techniques for the detection identification and phylogenetic analysis are based on the nucleotide sequence of 16S rDNA. This technique has been widely used for the study of microbiota of several dairy foods. Additional methods for the analysis of 16 rDNA amplicons are DGGE (Denaturing Gradient Gel Electrophoresis) and TGGE (Temperature Gradient Gel Electrophoresis), which have been demonstrated to be efficient tools for the analysis of complex microbial communities of cheese. These techniques allow the detection of species and population dynamics in complex microbial communities, without cultivation of bacteria. FISH (Fluorescent In Situ Hybridization) is an additional method, based on the microscopic evaluation of *in situ* hybridization with fluorescent probes targeted to microbial rRNA, which has been used for the study of bacteria in cheese.

In the last few years a collection of lactic acid bacteria was underwent to genomic sequencing and analysis. The genome sequencing of more than 15 different species, comprising the most interesting bacteria for dairy application, is under analysis or has been completed. The information deriving from the efforts to determine the genome sequence of a representative collection of lactic acid bacteria provide new possibility for the biodiversity analysis of dairy microorganisms. The new approach of DNA arrays gives a wide range of information on gene expression allowing a function analysis of dairy lactic acid bacteria. MLST (Multi Locus Sequence Typing) is a recent developed technique, based on the analysis of allelic variation of several different genetic loci, which allow determining the relatedness between the different analysed strains.

The knowledge deriving from the application of these innovative methods will permit a better understanding of the role of microbial biodiversity on the quality and the safety of dairy products.

Authenticity of dairy products

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Milk has been an important traded commodity since historical times and hence may be one of the earliest commodities to be subject of extension or adulteration. Moreover, dairy products continue to be a major challenge to modern food chemists. Challenges in the adulteration include the substitution of valuable compounds in milk products for less costly ingredients as well as label misinterpretation of dairy products. Food authenticity comprises several issues, e.g., cheating consumers, unfair competition to legitimate industry, damage to consumer perception of products and burdens on regulatory authorities. Thus, in controlling food authenticity, several aspects have to be considered, e.g., the substitution or omission of compounds, addition of ingredients to make products appear to be better, the identity and false or misleading labelling of products. Although nutrient quality may be an issue (e.g., allergy to cows' milk), the identity of dairy products is usually not an issue of safety. However, as economic adulteration and branding is prohibited, food chemists and control authorities are challenged to keep abreast of recent developments in the dairy industry.

The intentional adulteration of milk and dairy products mostly covers the following aspects:

- ▲ the non-compliance of the product with the standard requirements (including the classification or labelling) (e.g., addition of water, skimming of fat, neutralisation),
- ▲ the use of unexpected or not allowed ingredients, extenders or additives, and
- ▲ the use of unexpected or not allowed treatments or technological processes.

As there are an immense number of possible frauds within these issues associated with respective analytical methods of detection, a comprehensive review would be very extensive. Therefore, authentication of dairy products is to be exemplified by focusing on several relevant (and the related modern analytical techniques):

- ▲ milk from different species (species identification by electrophoresis, HPLC and PCR)
- ▲ milk protein polymorphism (detection of genetic variants of milk proteins in promoted "using isoelectric focusing and PCR; identity control of individual cows)
- ▲ type, degree of ripening and/or age of cheese (e.g., electrophoretic ripening index for the determination of proteolysis and the deduction of the age of Parmesan cheese)
- ▲ geographic origin of milk and dairy products (e.g., milk powder and PDO cheeses)
- ▲ effects of processing (e.g., cheese from UHT milk)
- ▲ effects of thermal treatment (types of market milk: raw, pasteurised, UHT, sterilised milk powder in milk and cheese; natural versus processed cheese; casein/caseinate in milk & cheese)
- ▲ casein/whey protein ratio (protein standardisation of liquid milk products; fresh cheese)
- ▲ milk powder composition (buttermilk solids and/or rennet whey solids in milk powder)
- ▲ non-dairy fats in butter (substitution for fats and oils of animal/vegetable origin)
- ▲ differentiation of rennet types (rennet's of animal, vegetable or microbial origin, recombinant chymosin)
- ▲ identification of lactic acid bacterial strains (e.g., strains with probiotic relevance).

Thus, authenticity control of dairy products remains a major challenge to dairy scientists!

Microbial diversity in traditional Italian cheeses

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Among the factors that link the typicality of a cheese to the cheese producing area, the importance and role of both raw milk- and environmental-microflora should not be underestimated. Non-starter lactic acid bacteria (NSLAB) are considered important micro-organisms for the development of cheese flavour. Previous investigations on cheeses with Protected Designation of Origin (PDO) suggest a probable specificity of the flora according to the origin of the milk.

This paper will present preliminary results (first-year activity) of a subject-oriented project funded by the Italian Agriculture Ministry and entitled "Exploitation and preservation of the peculiar (autochthonous?) microflora of Italian cheeses". This project, to which participate 19 Italian laboratories and universities, is aimed at answering this basic question: are raw milk- and/or environmental-microflora key factors linking the typicality of a cheese to the cheese production area?

To this end, we have been studying 44 (essentially raw milk) cheese typologies produced from cow, ewe, goat, and buffalo milks in 18 different Italian regions. The project is divided into two sub-projects covering two basic themes: A - building up a collection of micro-organisms typical of regional Italian cheese productions; B - evaluating the role of the natural whey starter culture as a possible link to the cheese production area in Grana Padano and Parmigiano Reggiano cheeses.

Concerning the sub-project A, approx. 6500 NSLAB have been isolated, identified and partially subtyped by polyphasic methods. The majority of the strains belonged to the *L. casei* and *L. plantarum* groups, *L. rhamnosus*, *L. curvatus*, *L. brevis*, *E. faecium*, *S. thermophilus*, *S. macedonicus*, *Lact. lactis* subsp. *lactis*, *Lact. lactis* subsp. *cremoris*, *Pd. pentosaceus*, *Pd. acidilactici*, *Leuc. mesenteroides*, and *Leuc. lactis*. Within the sub-project B, about 700 thermophilic LAB belonging to *L. helveticus*, *L. delbrueckii* (subsp. *lactis*), *L. fermentum*, and *S. thermophilus* and 80 lytic bacteriophages were isolated from 50 whey starter cultures. Strains, which were subtyped essentially by RAPD-PCR, highlighted wide diversity, e.g. different ecotypes from Grana and Parmigiano were shown.

Future work: For a better strain comparison and biodiversity estimation and to dispose of a really useful strain Collection, future work will be devoted to interlaboratory data homogenisation using already available bio-informatics tools (e.g. BioNumerics).

Perspectives: How to use this microbiological richness? Could these micro-organisms be used as starter or starter adjuncts? In the dairy industry, the use of starters, on the one hand, has been positive with respect to the quality of the product, but, on the other hand, it has diminished the diversity of fermented dairy products. Since the dairy industry is keen to explore new possibilities for increasing product range diversity, there would be interest in searching for potential new starter organisms from this pool of NSLAB. Therefore, a reduced number of strains, selected on the basis of biodiversity estimation, will be characterised by looking at phenotypic properties of technological interest (e.g. starter functions, role in flavour formation, and role in cheese ripening, ecological aspects ...).

The geographical determination of dairy products using the stable isotopes of life

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Within the last years, the stable isotope method proved its reliability in the determination of the geographical origin of wine and led to an established EU-database.

Dealing with the fact that there is a well-known pattern of D/H and ¹⁸O/¹⁶O in the meteoric water as well as in the ground water, every material including plants and animals gets a significant fingerprint in the tissue water depending on their region.

If the water has been changed or lost by drying, the organically bound forms of oxygen and hydrogen serve as indicators as well. The pattern of D/H and ¹⁸O/¹⁶O in the meteoric water depends mainly on the temperature, the landscape altitude and the distance from the sea (continental effect).

Adding the remaining stable isotopes of the elements of life (C, N, S) further information is available: Soils show different isotope ratios of ¹⁵N/¹⁴N and ³⁴S/³²S depending firstly on the natural geological composition and secondly on the cultivation. For example the isotopic composition of fertilizers varies from their type (organic nitrogen or synthetically produced from air nitrogen, sulphur of volcanic or biological origin). Plants implement these isotope ratios in their organic tissue which will be transmitted to animals as fodder.

Finally, there is an existing link in the food chain from the fodder to the cattle and to the milk as well. The ¹³C/¹²C isotope ratio depends on the type of metabolism and of local climatic influences as water stress under heat. Drawing a conclusion plants as maize (C4-plants) show different ¹³C/¹²C ratios to wheat (C3-plants). Therefore it is possible to determine the nutrition composition in cattle's tissue and in the milk product.

In the last 5 years the reliability of the stable isotope method was shown in cooperation with the German custom authority in tracing back the geographical origin of butter, as there was a suspicion of subsidy fraud.

Today, milk powder is included in the determination of the geographical origin as well.

Sensory analysis and quality management of typical dairy products

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Introduction

The word "typical" define a dairy product characterised of high level preferences both in time and space dimensions. The human preferences must link to sensory perceptions and these change through the time and space. "Typical" characteristics are strictly related to the dynamic of consumer preferences, the evolution of cheese quality definitions through the years is well known. The best way to measure and compute preferences and analytical characteristics of cheese is sensory analysis.

In modern quality management of dairy products sensory analysis is one the most important parameters to follow, but not the only one!

The aim of this work is to study relationships among technical, analytical and sensory properties (quality parameters) of Italian typical cheese.

Data set

There are three type of data set:

- ▶ sensory data set: analytical sensory profile and consumer preferences and perceptions;
 - ▶ chemical data set: most important quality parameters of cheese;
 - ▶ technical data set: quality management parameters of processes.
- It is possible to compute together the three set by means two type of "multivariate techniques" derived from family of factor analysis.

The technique permits the simultaneous (conjoint) analysis of the three spaces and the dynamics of interactions.

Results

Always statistical applications are not preferred by production, but it is the only tool to compute simultaneously large data set.

Numeric approach is confirmed by statistical graphics allowing more simple visual interpretations.

The study of interactions indicates the right way to follow to optimise market preferences and technical quality process in the restricted field of typical parameters.

The final goal is improving process control to optimise product typical perceived characteristics.

A global approach for the analytical traceability and authenticity of Emmental cheese

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Emmental or Swiss cheese is one of the most known cheeses worldwide and is produced in many industrialised countries at a very different price. To fight or prevent fraudulent labelling of cheese type, pertinent tools for geographic origin assignment are needed. For this purpose, cheese samples from the main European manufacturers were collected, corresponding to 110 winter summer samples. From a preliminary study, restricted to only 20 samples, a set of discriminant analytical methods was selected to investigate the 183 samples. These are: nitrogen fractions value, volatile fatty acids, salt content, organic acids, microorganisms (*Lb. helveticus*, obligate fermentative *Lb.*, enterococci), trace elements and isotope stable ratios. With the help of multivariate statistical analysis such as linear discriminant analysis, it was possible to assign the samples to the corresponding region, thus validating the preliminary choice of the analytical methods.

Application of multi-isotopic stable isotope ratio analysis to the geographical characterisation of North Italian cheeses

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Within the last few years, the multi-element stable isotope analysis has gained increasing importance in geographical characterisation of animal products like milk (Rossmann et al., 1998), butter (Rossmann et al., 2000), cheese (Manca et al., 2001) and meat (Plesentier et al., 2003). In this work traditional cheeses from North of Italy were studied in the framework of a project (MIROP 'Innovative methods for the traceability of origin and process to protect typical local dairy products'), funded by local government of Provincia Autonoma di Trento with the aim to characterise and prove the origin of 'Nostrani del Trentino' cheeses, using advanced and innovative methods such as multi-isotopic analysis.

Measurement of $2\text{H}/1\text{H}$, $13\text{C}/12\text{C}$, $15\text{N}/14\text{N}$, $34\text{S}/32\text{S}$ of casein, $18\text{O}/16\text{O}$ of glycerol by Isotopic Ratio Mass Spectrometry (IRMS) and $87\text{Sr}/86\text{Sr}$ of total material by Thermal Ionisation Mass Spectrometry (TIMS) was carried out on about 80 cheeses (Nostrani from Trentino, Asiago from Veneto, Toma from Piemonte, Montasio from Friuli Venezia Giulia, Fontina from Valle d'Aosta) produced in 2001.

$13\text{C}/12\text{C}$ resulted to be more linked to composition (corn content) of animal diet, $15\text{N}/14\text{N}$ to adopted agricultural practice (intensive or extensive), $18\text{O}/16\text{O}$ and $2\text{H}/1\text{H}$ to isotopic content of drinking water (local climate) and to presence of fresh grass in the animal food, $87\text{Sr}/86\text{Sr}$ and $34\text{S}/32\text{S}$ to geological characteristics of soil. These parameters all together can give an important geographical fingerprint of the different cheeses, improved by applying Multivariate Discriminant Analysis.

In details, cheeses produced on the mountains with milk from cows at pasture, present typical lower 13C and 15N content of casein and higher 18O of glycerol.

References

- Rodmann, A.; Kornel, B. E.; Versini, G.; Pichlmayer, F.; Lamprecht, J. *Food Sci. Nut.* 1998, 1, 9-21.
Rossmann, A.; Heberbauer, G.; Hözl, S.; Horn, P.; Pichlmayer, F.; Voerkellus, *Eur. Food Res. Technol.* 2000, 211, 32-40.
Manca, G.; Camin, F.; Coloru, G.; Del Caro, A.; Depontori, D.; Franco, M.A.; Versini, G. *J. Agric. Food Chem.* 2001, 49, 1404-1409.
Plesentier, E.; Valusso R.; Camin F.; Versini, *Meat Science* 2003, 64, 239-247

New super concentrated permeate process and Pure-LacTM concept

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APV's new super concentrated permeate process is based on instantaneous crystallisation and has benefits for the dairy industry. Instantaneous crystallisation removes the need for large crystallisation tanks. The Parafash unit requires only 50 % of the energy required by a spray dryer for equivalent throughput. Higher concentration and throughput plus lower energy costs mean faster return on investment. The plant requires less space, reducing conversion and/or building costs. Continuous crystallisation process permits more homogeneous crystallisation, avoiding many of the inconsistencies associated with crystallisation in batch tanks and the associated reduction in product quality.

APV's Pure-LacTM concept is efficient and innovative heat treatment process to obtain prolonged shelf life. Objectives of Pure-LacTM are reduction of critical spores by 8 decimals and protected freshness. Adds an extra "quality buffer" to the milk and is suitable for use with limited distribution lines. Sensory is similar to pasteurised milk, but has longer shelf life. It is cost effective and "tailored" to specific product requirements and enables branding. Useful is for the introduction of new products.

Chemical properties of Turkish Tulum cheese

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Tulum cheese is one of the most popular varieties of hard cheese manufactured in some provinces in the Mediterranean, Aegean and East Anatolian regions of Turkey. It has been classified as a hard cheese according to its total solid content. Tulum cheese is traditionally made from raw sheep's milk, sometimes from cow's milk or from a blend of cow's, goat's and sheep's milk. It has a sharp and piquant flavour. Lipolysis and proteolysis products such as free fatty acids and tyrosine respectively play a significant role on Tulum cheese flavour.

The objective of this work was to determine fat hydrolysis, degree of proteolysis, free fatty acid content and their effects on Tulum cheese flavour.

The chemical quality of the cheese was evaluated based on total solid, fat, salt, pH, titratable acidity, total nitrogen (TN), water soluble nitrogen (WSN), ripening index, tyrosine, acid degree value and total volatile fatty acids. Free fatty acids were quantitated in cheese by Gas Chromatography (GC) after their isolation from lipids and proteins.

Our results showed that the mean values for chemical properties of Tulum cheese were: 57.62 % for total solid; 2.69 % for titratable acidity; 4.57 for pH value; 51.64 % for fat in dry matter; 7.27 % for salt in dry matter; 3.34 % for total nitrogen; 0.59 % for water soluble nitrogen; 17.73 % for ripening index; 1.17 mg/g for tyrosine; 8.01 mg KOH/g for acid degree value; 31.55 ml 0.1 N NaOH/100 g for volatile fatty acids. The mean free fatty acid contents (mg/kg cheese) such as butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid and total free fatty acid were 750, 625, 733, 1846, 1536, 2717, 5608, 2210, 4999 and 21027, respectively.

These results suggest that among the chemical properties tested, salt in dry matter, ripening index, tyrosine value, total volatile fatty acid, acid degree value and free fatty acids were found very effective on the flavour.

Determination of origin of Slovenian cheese from chemical composition

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Within the context of foodstuff analysis, the study of volatiles and semi-volatiles, preferred their ease of sampling and amenability to automatic instrumental analysis, provides a representative set of chemical markers that characterize the whole process of production, seasoning and suitability for consumption. A relatively broad group of classes of compounds needs to be monitored in sample profiles must be compensated for varieties that include seasonal changes in animal products from various farms that produce under the same trade name. Thus, the determination of chemical composition in the production of cheeses serves multiple functions. It provides an easily accomplished chemical profiles to all production steps. This quality information serves as a basis for any evaluation, including identification of source, identity, and quality. It also provides a framework for evaluation of possibly controversial health related issues. It promotes knowledge and understanding of the role of subtle variations especially in artisan cheese production facilities and may assess the robustness of production practices within the region. It provides scientific ground for legal steps in the process of obtaining and enforcing the Protected Designation of Origin (European Council Regulation (EEC) No. 2081/92), which may be a valuable component of an overall market strategy in the context of declining European cheese sales. Finally, it serves as a starting point for any down-streamed - simplified monitoring procedures, like those using 'electronic nose' methods coupled with chemometric and artificial intelligence (AI) data profiling and/or data mining.

Three national cheeses under consideration for the Protected Designation of Origin (PDO European Council Regulation (EEC) No. 2081/92), two hard cheeses 'Tolminski sir', 'Nanoski sir' and a soft 'Mohant' cheese were studied. VOC profiles were determined by dynamic headspace with off-gassing and purge and trap technique combined with Solid Phase Micro Extraction (SPME). On the other hand, the composition of fatty acids was monitored by in-situ derivatization and subsequent analyses. The chemical composition was correlated with cheese origin by using different chromatographic and AI techniques.

The traditional cheesemaking of Kachaval originated from Stara planina

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Nomads from Greece implemented the tradition of Kachaval cheesemaking process on Stara Planina, 100 years ago. At the beginning, the Kachaval was made exclusively from ewe's milk, during the grazing period. In the past, Kachaval was made on «bačijak» and besides the Kachaval the significant by-products were ghee and «urda». The historical data showed that in 1903, 160 wagons of Kachaval were exported in Vienna and Budapest. The changes in cattle-breeding conditions lead to significant reduce in number of ships, and consequently caused the discontinuance in production of Kachaval. Nowadays, there are few dairies-Bačija on Stara Planina which carrying out the traditional way of Kachaval production. The characteristics of chemical content of traditionally made Kachaval are following: % dry matter 60.79 ± 3.37 , % milk fat 29.50 ± 2.25 , % moisture on a fat-free basis 55.57 ± 3.55 , % fat in dry matter 48.52 ± 2.41 , % proteins 24.99 ± 1.60 , % NaCl 3.8 ± 2.57 , pH value 5.62 ± 0.09 .

The traditionally made Kachaval have shape of flatten cylinder, with weigh 6-7 kg, bright-yellow rind and firm dough. The traditional cheesemaking of Kachaval compromise the hand - made process of "pasta filata" cheese. Revitalizing the traditional Kachaval production also compromise the preservation of cultural identity of region of Stara Planina.

Key words: Kachaval, Stara Planina, traditional cheesemaking

The "Sirac" - contribution to the technology knowledge and quality of traditional Bosnia and Herzegovina cheeses

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The *Sirac* is traditional cheese of Bosnia and Herzegovina, produced in area of Tomislavgrad town. The taste is mild and moderately salt. It is consumed fresh, immediately upon production, or mature, after ripening. The technology is specific and according to that the *Sirac* can be classified as acid coagulated cheese. Milk proteins are coagulated by use of high temperature and acidity without adding of rennet. The aim of the work was to investigate traditional technology of the *Sirac* in small village households and to establish optimal technology. The ten tries in laboratory condition were done. The chemical composition of cheese milk, whey, fresh and ripen cheese were determined. During experiments all steps in technological process were followed. Special attention was paid to temperature and acidity of milk coagulation, draining of whey and pressing. Cheese was sensory evaluated after ripening period. The quality of experimental cheese was high. Average content of moisture was 37.03 , fat in dry solids 47.01 , protein 28.39 and ash 3.80 percentages. The sensorial quality was good. Average score for all ten cheeses was 18.3 points. The yield ranged from 12.25 to 17.76 and showed that distribution of components to cheese was high. The analyses of whey confirmed this. Fat and protein content of whey were 0.58 and 0.43 percentages. Due to obtained results it can be concluded that standard technology of the *Sirac* can be set up and make basis for industrial production.

Characterization of autochthonous lactic acid bacteria from artisanal Italian cheese

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In recent years several studies have been conducted to select from raw milk and artisanal cheeses produced without starter cultures autochthonous bacteria. Increasing information on the natural microbial population present in dairy products can help to prevent the loss of microbial biodiversity in typical foods and consequently the loss of a wide range of cheeses produced by different methods whose typical features depend on local and regional traditions and on the indigenous microbial population present in raw milk and selected by the cheese-making environment. Moreover these studies could be the basis for the selections of new strains to be used either as specific cultures in a large-scale production of traditional cheeses improving the existing dairy product manufacture. Indeed the diversity of starters used in industrial dairy fermentation is low and there is an increasing demand from cheese makers for new starter that show advantageous effects on cheese characteristics. Then the aim of this work was to study the natural lactic bacteria population of an artisanal Italian cheese from Piedmont (Northern Italy) and produce a new starter to be used in a large-scale production. Isolates collected from curd and ripened artisanal cheeses were identified by the combined use of PCR 16S-23S rDNA spacer analysis, species-specific probes and 16S rDNA sequencing. Lactococci constituted 67 % of the coccal isolates. Enterococci were also isolated together with strains of *Streptococcus macedonicus* and *S. thermophilus*. Lactobacilli were only detected in three samples of curds. For each isolate was also determined the acidification and the proteolytic activity and the aroma production by the SPME-GC-MS. On the basis of obtained results some isolates were selected and about twenty starters used in cheese-making with raw and pasteurised milk were produced. The obtained cheeses were evaluated with sensory analysis and two showed good results with typical taste and flavour. The results suggest the possibility to use these starters in dairy industry as new strains and in artisanal cheese-making to improve the existing cheese quality.

Tosèla di Primiero cheese: influence of a *Streptococcus macedonicus* and *Lactobacillus paracasei* natural integration culture on cheese flavour

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Tosèla di Primiero is a traditional, non ripened fresh cheese produced from pasteurised milk collected in the Paneveggio-Pale di San Martino alpine area belonging to a Natural Park in the Italian Dolomites. According to the traditional cheese making technology, the used milk should be raw whole milk, but the safety issues due to a broader distribution of the product and the need of a lengthened shelf-life imposed in the recent times the employment of pasteurised milk. This led to a more flat and poor taste and flavour of this cheese, no more recognized as the traditional one. The aim of this work was to recreate in the pasteurized milk used to the Tosèla cheese making, a composition of typical lactic acid bacteria isolated from the raw milk in order to contribute to the development of original sensorial properties of the cheese, moreover to allow its longer conservation. The microbial composition of the raw milk utilized for the Tosèla cheese making before the pasteurisation is described. The most represented lactic acid bacterial strains were isolated and characterized, two of them were selected to inoculate the milk after the pasteurization in experimental cheesing processes. The bacterial mix was composed by two strains belonging to the *Streptococcus macedonicus* and *Lactobacillus paracasei* species, selected for their low-acidification and flavour development ability, respectively. We followed the inoculated strains by means of molecular methods (PCR) after 3 days of cheese ripening, and a panel of judges tasted the produced cheeses, comparing the inoculated ones to a standard production. The two strains became the dominant population in inoculated cheese up to 3 days, while the control cheeses were populated by a broad variety of acid bacteria probably originating from the adventitious microbiota present on the dairy surface. Inoculated cheeses were appreciated by the judges more than the standard for their sensorial properties. The employment of a natural culture composed by indigenous strains could be a way to contrast adventitious microbiota present in the milk and to contribute to the flavour development in Tosèla cheese, saving the traditional properties of this product.

Alpine environment microbiota influences the bacterial development in traditional raw-milk cheese

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Nostrano di Primiero is a six month ripened cheese produced from raw milk collected in the Paneveggio-Pale di San Martino Natural Park in the Italian Dolomites. In summer this cheese is made using milk collected from two different areas, Passo Rolle and Vanoi, in the Paneveggio Natural Park. During the experiment the milk from the two areas was separately processed and cheeses were made in the same cheese factory using the same technological process. The microbiota of raw milk and cheeses of the two areas was isolated and the dominant population was monitored by RAPD analysis and identified by 16S rRNA sequence. The milk of the Passo Rolle area was mainly composed of mesophilic strains, thermophilic *Streptococcus thermophilus*, and low amounts of enterococci were also found; the milk of the Vanoi area was dominated by mesophilic microbiota mostly *Lactococcus lactis* ssp. *cremoris* and ssp. *lactis* and *Lactobacillus paracasei* ssp. *paracasei*. The plating of natural starter culture revealed the presence of a relevant community of thermophilic cocci and lower amounts of enterococci. The dynamic population analysis showed the importance of natural starter culture in the first two days of cheese ripening in both cheeses. Moreover the large biodiversity observed in the raw milks was also detected in the cheeses during ripening. The Vanoi cheese which was dominated by *Enterococcus faecium* and *Str. macedonicus* in the first two days and mesophilic *Lactobacillus paracasei* ssp. *paracasei* became the most represented population after 15 days of ripening. In the first days the Rolle cheese was characterized by being mainly composed of thermophilic *Str. macedonicus* and *Str. thermophilus* and secondarily by mesophilic cocci. During ripening the microbiota composition changed since at 15 days the mesophilic lactobacilli was the dominant population but later this was mainly composed of mesophilic cocci and lactobacilli. The taxonomical identification by 16S rRNA sequence confirmed a large biodiversity related to raw milk microbiota and only five strains of *Str. macedonicus*, *Lb. plantarum*, *Lactobacillus paracasei* ssp. *paracasei*, *Lb. fermentum* and *E. faecium* were detected in both cheeses.

Microbial diversity of non-starter lactic acid bacteria in Italian artisanal cheeses

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Among the factors that link the typicality of a cheese to the cheese producing area, the importance of the role of both raw milk and environmental microflora should not be underestimated. Most raw milk micro-organisms are mesophilic, nonstarter lactic acid bacteria (NSLAB), mainly belonging to *Lactobacillus* spp., *Pediococcus* spp. and *Enterococcus* spp. The NSLAB are considered important microflora for the development of cheese flavour. To this regard, previous investigations on Camembert and Comté cheeses with Protected Designation of Origin (PDO), suggested a probable specificity of the flora according to the origin of the milk (1-3).

The aim of this work was to comprehend the technological role of NSLAB isolated from Italian cheeses. A microbial characterization of about 300 strains isolated from different Italian, artisanal cheeses were then carried out. Genotypic identification and typing were performed by RAPD-PCR using different oligonucleotide primers. RAPD-PCR dendrograms were deduced by UPGMA using Pearson correlation coefficient. DNA sequencing of partial 16S rRNA gene was carried out on unidentified strains. Phenotypic characterization was carried out by classical biochemical and physiological tests.

Most NSLAB belonged to *L. casei*, *L. plantarum*, *L. rhamnosus*, *L. zeae*, *L. brevis*, *L. curvatus*, *E. faecium*, and *E. faecalis*. RAPD-PCR evidenced wide strain heterogeneity, due to the various isolation sources (raw milk, milk and cheese production environments). Strains within different species studied here were grouped on the basis of the different cheese types.

In the same study, about 200 starters LAB belonging to *Lactococcus lactis* and *S. thermophilus* were isolated as well. Strain differences within isolates studied here would allow better understanding the ecological relationships among microorganisms and comprehending the role of atypical strains, which are often isolated from cheese. According to Wouters *et al.* (4), we suggest a possible use of NSLAB as adjunct cultures. To this end, both phenotypic and biochemical characterization will be carried out on isolated strains.

References

1. Bizzarro R., Torri Tarelli G., Graffia G. and Neviani E. Phenotypic and genotypic characterisation of lactic acid bacteria isolated from Pecorino Toscano cheese. Ital. J. Food Sci. 12, 303 (2000).
2. Bouton Y., Guyot P. and Grappin R. Preliminary characterization of microflora of Comté cheese. J. Appl. Microbiol. 85, 123 (1998).
3. Coroller D., Mangin I., Desmasures N. and Gueguen M. An ecological study of lactococci isolated from raw milk in the Camembert cheese registered designation of origin area. Appl. Environ. Microbiol. 64, 4729 (1998).
4. Wouters J.T.M., Ayad E.H.E., Hugenoltz J. and Smit S. Microbes from raw milk for fermented dairy products. Int. Dairy J. 12, 91 (2002).

Mohant cheese - unique from the raw milk to the consumer

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Strong smelly semi-soft mountain cheese, called Mohant, originates from Bohinj region, which is settled in the bosom of the Julian Alps. Geographically separated area enabled the formation of a unique cheese, typical in its production, taste, smell and texture. Since 1997 different activities have been taking place with the intention of preserving the originality of the Mohant cheese. Mohant cheese is traditionally manufactured from the raw milk of the autochthonous cow breed "bohinjska čika". After curdling the milk with the addition of rennet, the coagulum is cut and the whey is removed. The drained curd is transferred into containers, called "deže", where it is first salted, then pressed and finally weighted. Cheese in "deže" is then fermenting for 12 hours at 20-22 °C, causing the remaining whey to excrete, which leads to the formation of anaerobic conditions. Fermentation is followed by the three step ripening: the cold ripening at 12-16 °C for 15 up to 20 days, the warm ripening at 19-23 °C for 15 up to 20 days, where cheeses undergo the most drastic changes in texture and taste, and the concluding cold ripening at 12-16 °C for up to 2 months.

In our work we focused on getting preliminary information on the predominant microbial population of Mohant cheese. The results could be beneficial in two areas. Namely, we could make accurate microbial specification and characterization of Mohant cheese, and moreover, we could prepare the starter consisting of "natural" isolates from Mohant cheese suitable for its production on a commercial scale.

Samples of matured Mohant cheese were collected from 6 different Mohant producers and aseptically prepared for microbiological analysis. In order to select most of the lactic bacterial species present in the samples, appropriate selective media were used as follows: CATC agar for enterococci, Rogosa agar for lactobacilli and M17 agar for lactococci. *Enterobacteriaceae* were grown on EMB agar and coliforms on VRB agar; clostridia were grown on SPS agar and proteolytic bacteria on milk agar. After incubation, 10 colonies were randomly picked from the countable plates (CATC, Rogosa or M17 plates, respectively, purified and submitted to Gram staining and catalase test. Growth of the coccal-shaped organisms was examined in M17 agar containing 20, 40 and 65 g NaCl/l and at 10 and 45 °C. The rod-shaped strains were tested for homo/heterofermentation. Isolates from Rogosa and CATC, respectively, were further analyzed to rod level by the PCR reaction.

In all 6 Mohant cheese samples lactobacilli counts varied between 3×10^7 - 1.9×10^8 cfu/g of cheese. Lactococci were determined in range between 2.1×10^7 - 9.7×10^8 cfu/g of cheese, while enterococci were determined in numbers of 1×10^5 - 1×10^7 cfu/g of cheese. The proteolytic isolates were present at a relatively high numbers as well (up to 5×10^7 cfu/g of cheese). All isolates tested were catalase negative and only CATC isolates (presumably enterococci) were able to grow at the presence of 65 g NaCl/l. The presence of some *Enterobacteriaceae* and coliforms was detected, but no clostridia were observed (in 1 g of cheese). PCR reaction confirmed all Rogosa isolates as *Lactobacillus* members and all CATC isolates as *Enterococcus* members.

This study provides baseline data on the predominant microflora of Mohant cheese which indicates basically a lactic fermentation process with probably significant role of proteolytic bacteria that influence the aroma and texture of the final product.

Overview of microbial population and identification of enterococci during autochthonous ewe's cheese ripening

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Autochthonous ewe's cheese - Kraški ovčji sir is cheese with protected denomination of origin (PDO). It is a starter-free farmhouse cheese, made from raw ewe's milk. Like in other PDO cheeses from Mediterranean region, enterococci represent the predominant lactic acid microflora, which can be very heterogeneous. They are responsible for typical fermentation and aroma development; on the other hand, some of them are nosocomial pathogens and carriers of genetic determinants for antibiotic resistance. Their ambiguous nature obliges us to identify them correctly and to assess their role in cheese ripening. The first step was made in this research. Additionally, an overview of other lactic acid bacteria (LAB) and coliform microorganisms was performed. Samples from two batches of cheese were taken in all steps of cheese-making process, beginning with milk samples, cheese samples before and after 24 hours in brine and then every 14 days until the end of ripening (60 days). CFU from M17 for LAB and VRB selective media for coliform bacteria were enumerated throughout the process. CFU from selective agar-media for enterococci KAA (Kanamycin Aesculin Azide agar) were enumerated as well and colonies were randomly picked from the plates. After purification, the isolates underwent Gram staining, microscopic examination and biochemical tests, including growth on the presence of 2, 4 and 6 % NaCl and 40 % (wt/vol) bile. Growth at 10 and 45 °C was observed. In the next step, the isolated strains were identified by PCR reaction with the use of genus-specific oligonucleotide primers in order to distinguish between enterococci and lactococci. For this step, another PCR reaction was performed, this time with species-specific oligonucleotide primers for the final identification. Based on the results, we can conclude that the predominant microflora in Kraški ewe's cheese consists of *Enterococcus faecalis* and that the number of CFU on selective agar media is spanned from 1.5×10^4 CFU/ml in milk to 1.7×10^8 CFU/g of 60-days-old cheese. The number of coliform bacteria decreases continuously from 7.4×10^7 CFU in cheese after salting and is absent in 1 g of cheese after 45 days of ripening. The numbers from MRS and M17 media were 1.7×10^8 CFU/g of 60-days old cheese and 9.0×10^7 CFU/g of 60-days-old cheese, respectively.

Mineral value of artisanal cheeses produced from raw sheep's milk in the Mediterranean area of Croatia

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The purpose of the present research was to estimate the concentrations of Ca, P, K, Mg, Mn, Zn, Cu and Fe in artisanal hard cheeses made from raw sheep milk in the different Mediterranean areas in Croatia. The mineral element content of cheese is of nutritional significance for humans; therefore it is very important to know its exact value in order to evaluate the contribution of cheese to diet.

Unfortunately, the mineral composition of cheeses is characterised by extreme variability due to different composition of milk, genetic, physiological and environmental factors. In particular, the number of scientific publications dealing with the data on mineral elements related to sheep's cheeses made in traditional ways is rare. No research on mineral content has been performed on artisanal cheeses in Croatia. Therefore this study was of interest both from nutritive and a geographical indication.

Forty two samples of hard artisanal farmhouse cheeses made from raw sheep's milk were randomly collected over the period of two years. The cheeses were divided, according to their origin, into three groups: cheeses from the Istrian peninsula ($n=13$), from the island of Pag ($n=17$) and cheeses from the other Dalmatian islands ($n=12$). They were manufactured from February to June, and at the moment of analysis each cheese was in two-month aging period. All cheeses were made from a mixture of evening and morning milking without the addition of starter or non-starter cultures. The cheese samples were grated and then analysed. The pH, dry matter and fat content (g/100g cheese) were determined according to FL-IDF 4A: 1982; 152:1991 methods. The concentrations of Ca, K, Mg, Mn, Fe, Zn and Cu of wet sample (mg/100g) were estimated by atomic absorption spectrometry, using a PU-9100X ASS spectrophotometer (Pyeunicam, UK). The determination of P was carried out using PU 8600 UV/VIS spectrophotometer (Pyeunicam, UK) at 420nm (yellow complex).

Mean concentration found in cheeses for Ca, P, K, Mg, Mn, Fe, Zn and Cu according to their origin were: Istrian peninsula 686.61, 521.53, 82.53, 33.20, 0.0158, 0.9030, 3.492, 0.0243; island of Pag 698.58, 566.12, 99.10, 34.65, 0.0152, 1.0594, 3.900, 0.0265 and Dalmatian islands 726.66, 567.33, 100.88, 30.68, 0.0098, 1.0391, 4.0166, 0.0488 mg/100g cheese, respectively. Significant difference between areas was estimated only for K, Fe, and Zn concentration.

The correlation between minerals and dry matter, fat and pH for 42 samples of cheese showed: high correlation between Ca and P ($r=0.909***$), K and Zn ($r=0.698***$); these minerals were correlated with fat (r approx. 0.5**) and dry matter ($r>0.7***$).

The value of the determined macrominerals (Ca, P, K, Mg) and trace elements (Mn, Fe, Zn, Cu), independently of the origin, in all cheeses were high and within the ranges according to the literature data. On the nutritional standing, however, the main value of those artisanal cheeses is in their rather high concentration of Zn.

Significantly lower concentration of K, Fe and Zn was found in Istrian cheeses, while the concentration of other minerals was area independent. It could be concluded that those minerals may be considered as a useful criteria for distinguishing the Istrian cheeses from the Dalmatian and Pag cheeses regarding the geographical indication.

Microelement composition of north Italian typical cheeses

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The mineral composition of the dairy products has been frequently investigated with several aims, in particular to focus some technological aspects, e.g. the differentiation of the source of milk (1) or the manufacturing processes (2, 3), and to define the contribution to the human diet (4, 5). More recently, micro- and trace-elements, and their isotopes (light-isotopes), have been used to characterise and protect the geographical origin of milk and cheese (6).

Today, the availability of multielement analytical techniques like ICP-OES and ICP-MS allows a faster and more complete description of the mineral composition of these products.

To survey the production of the typical cheeses of its area, the local government of the Provincia Autonoma di Trento funded a specific research project named "MIROP" ("Innovative methods for the traceability of origin and process to protect typical local dairy products").

In this paper we present the mean composition of six Trentinian type of cheeses (Spressa, $n=9$; Puzzone, $n=10$; Vezzana, $n=8$; Nostrano di Primiero, $n=8$; Nostrano Val di Sole, $n=8$; Nostrano Castelfondo, $n=8$) and that of other four important type of cheeses from North Italy (Montasio, $n=8$; Toma, $n=8$; Asiago, $n=8$; Fontina, $n=8$).

The possibility to discriminate the cheeses on the basis of their geographical origin is presented.

References

1. Fresno, J. M.; Prieto, B.; Uribeles, R., and Sarmiento, R. M. *J Sci Food Agric.* 1995; 69: 339-345.
2. Coni, E.; Bocca, B.; and Caroli, S. *J Dairy Res.* 1999; 66: 589-598.
3. De la Fuente, M.A.; Ollano, A.; Casal, V.; and Jurez, M. *J Dairy Res.* 1999; 66: 65-72.
4. Coni, E.; Allimonti, A.; Bocca, A.; La Torre, E.; Pizzuti, D.; and Caroli, S. *Element Speciation in Bioinorganic Chemistry*; Wiley and Sons, Inc. 1996; 255-284.
5. Brätter, P.; Blasco, I. N.; Negretti de Brätter, V.E.; and Raab, A. *Analyst.* 1998; 123: 821-826.
6. Versini, G.; Camin, F.; and Deperiori, D. *Statistical evaluation of light-isotope and multi-element data to verify the geographical origin of milk and cheese -preliminary results- Annual progress report 2 EU project SMT - CT98-2236, 2001.*

Evaluation of experimental procedures for processing Marajoara cheese manufactured from buffalo milk *

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Marajoara cheese is a kind of soft cheese manufactured from buffalo milk in the Northern regions of Brazil. Cheese is often artisanally manufactured in farms and technological procedures are not standardised or well understood. Besides, variations in the quality of the product are greatly influenced by microbiological characteristics of raw milk. Therefore, the aim of this study was to evaluate different technologies for the manufacture of Marajoara cheese, using four methods applied to the buffalo milk: T_1 - natural acidification, T_2 - acidification with citric acid, T_3 - pasteurisation at 65 °C for 30 min; and T_4 - pasteurisation at 71 °C for 5 min. Cheese produced was stored for 30 days. Samples were collected on days 1, 15 and 30 and submitted to the following analyses: coliform, mesophile and psychrotrophic counts (1), determination of pH, fat and protein content, total solids and non-fat solids (2), texture and sensorial evaluation (3). Results were submitted to statistical analysis by one-way ANOVA. Mesophile and psychrotrophic counts were relatively low in all treatments (mesophile: $< 4.71 \text{ Log CFU/g}$; psychrotrophic: $< 4.06 \text{ Log CFU/g}$). T_3 and T_4 cheese had lower mesophile counts ($P < 0.05$) on day 1; however counts increased after day 15 of storage. Compared with other treatments, T_1 cheese presented higher psychrotrophic counts ($P < 0.05$). Total and fecal coliforms were not detected in cheese samples. Besides, there were not significant differences ($P > 0.05$) for fat, protein and yield among treatments. On day 1, acidity was higher ($P > 0.05$) on T_2 cheese. On day 15, acidity of T_1 , T_2 and T_3 increased and were higher than T_4 . Texture parameters (TPA hardness, TPA, TPA chewiness and TPA gumminess) were lower in T_1 and T_2 cheese on days 1 and 15. Differences in TPA springiness values were not significant ($P > 0.05$) between the treatments, and no variations occurred during storage at 5 °C. Sensory evaluation revealed higher final scores ($P < 0.05$) for T_1 and T_2 cheeses, whereas T_3 presented the lowest score. It is concluded that heat treatment as used in the present experiment decreased the overall quality of Marajoara cheese, hence indicating the need for new approaches for the manufacture of this product in order to preserve original characteristics and prevent microbiological risks.

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References

1. American Public Health Association, 1992, *Standard methods for the examination of dairy products*. 18th ed. Washington: APHA.
2. Association of Official Analytical Chemists, 1990, *Official Methods of Analysis*. 15th Edition. AOAC, Arlington, VI.
3. Institute of Food Technologists, 1981, *Sensory evaluation guide for testing food and beverage products*. Food Technology, 35: 50-59.

Effects of somatic cell counts (SCC) on cheesemilk composition and yield efficiency of artisan's Tounj cheese

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Production of autochthonous cheese on family farms in Croatia has been increasing very. The improvement has been a direct result of the actions under the project, "Investigation and Development of Technology of Autochthonous Cheese Produced on Family Farms". Regular hygienic control of raw milk for middle and large-scale dairy processing plants has been performed in Croatia since 2000, but the same control has not been established for milk processed at the family-farm level. Therefore, the objective of this paper was to investigate the effects of somatic cell count (SCC) on cheesemilk composition and yield efficiency of Artisan's Tounj cheese produced on family farms to emphasize the importance of regular control of raw milk quality at the farm level. The investigation was performed by weighing cheesemilk, whey and cheese (after smoking) of 30 batches of cheese produced on five family farms. During the investigation, samples of milk, whey and cheese were collected for laboratory analysis. The batches were divided according to SCC level into three groups: $< 100,000$ cells/ml; $100,000 - 1,000,000$ cells/ml; and $> 1,000,000$ cells/ml. The results showed that lactose content in cheesemilk significantly ($P < 0.05$) decreased from an average 4.66 % in low-SCC milk ($< 100,000$ cells/ml) to 4.41 % in high-SCC milk ($> 1,000,000$ cells/ml). Effect of solid non-fat content which significantly ($P < 0.05$) decreased from 8.72 % to 8.22 %. Titratable acidity also significantly ($P < 0.05$) decreased from 6.95 SH to 6.20 SH; while the free point significantly ($P < 0.05$) increased from -0.5229 °C to -0.5123 °C. Fat recovery was significantly ($P < 0.05$) lowered (10 %) in high-SCC milk which caused a significant ($P < 0.05$) loss of fat. Protein recovery and yield efficiency decreased as a result of high-SCC in cheesemilk, but these results were not statistically significant. Protein recovery decreased by 2.08 %, and yield efficiency by 4.04 %. Deleterious effects of high SCC in cheesemilk on its composition and yield efficiency were demonstrated; therefore, regular control of hygienic quality of raw milk on the farms for autochthonous cheese production is essential.

Key words: somatic cell counts, cheesemilk, fat and protein recovery, yield efficiency and Artisan's Tounj cheese

Profiling of volatile organic compounds in some Slovenian cheeses by purge-and-trap

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One of the possibilities to provide scientific background for legal steps in the process of obtaining and enforcing the Protected Designation of Origin (European Council Regulation (EEC) No. 2081/92) is determination of characteristic concentration profile of volatile organic compounds (VOCs) in individual cheeses. Three national cheeses, that is, two hard cheeses "Tolminski sir", "Nanoški sir", and a soft "Mohant" cheese were studied. Dynamic headspace technique with cryofocusing followed by GC/MS analysis was used to obtain information on volatile organic compounds, which represent respective chemical fingerprint of these cheeses. With dynamic headspace purging, all classes of volatile and semi-volatile compounds are encompassed. VOCs are purged by helium to cryotrap attached to gas chromatograph. By switching 10-port valve and heating the cryotrap, organic compounds are injected in to capillary column. Since the mentioned cryofocusing step is largely non-selective, a genuine concentration profile of these compounds may be obtained.

Several classes of volatile organic compounds were identified, including aldehydes, ketones, alcohols, acids, and terpenes. Reasonably good inter-run reproducibility of relative concentration profiles was found, though the overall intensities varied, due to variations in recovery. Compounds and compound classes were studied as to their suitability for serving as markers of the origin of these cheeses. Specifically, terpenes that come from animal diet and proceed chiefly unaltered through production steps to the final product were identified and assessed as appropriate for this purpose. It is important in the process of specification to know the broad spectrum of specific chemical features to guard the original products from adulteration.

d.

CAP Reform, WTO and EU Enlargement - Challenges for the Dairy Industry

Use of terpene and sesquiterpenes for mountain cheeses characterisation

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Terpenes are volatile compounds originating exclusively from plants that can be recovered in dairy products. In fact these terpenes are readily recovered in milk fat thus making possible to trace the diet of dairy animals. Several studies have therefore been conducted to use the terpenes to trace the geographical origin or the nature of the feed supplied to animals. Results have showed that the milk from different production sites (lowland vs. highland) and seasons (winter vs. summer) can be distinguished. Our aim was to determine if the terpene profile can be used to distinguish among mountain pastures with different vegetation types resulting in different forage types. The terpenes and the sesquiterpenes of ricotta cheese samples produced during summer in three mountain farms and the sesquiterpenes of ricotta cheese samples produced during summer in three mountain farms were determined with SPME-GC-MS. The ricotta cheeses were produced with cow and goat milk and samples were analysed every three days. In all samples high quantities of terpenes (over all α -pinene, β -pinene, camphene, p-cymene, β -myrcene and limonene) were determined. Several sesquiterpens as α -caryophyllene, α -copaene and 9-epicaryophyllene were identified too. These compounds showed a wide variability between the samples due to stage of plant development but discrimination among the farms still was highlighted with the Linear Discriminant Analysis and the Artificial Neural Network. Hence terpenes and sesquiterpenes can be used not only to distinguish between summer pastures cheese and the one produced during other periods but also as markers of each mountain pasture.

Profiling of volatile organic compounds in some Slovenian cheeses by SPME technique

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Three national cheeses under consideration for the Protected Designation of Origin (PDO European Council Regulation (EEC) No. 2081/92), two hard cheeses "Tolminski sir", "Nanški sir" and a soft "Mohant" cheese were studied concerning the characteristic content of volatile organic compounds (VOC). The volatiles were sampled from the aliquot of grated cheese by purging with helium and pre-concentrated in a specially designed cryotrap. After equilibrating to ambient temperature the volatile organic compounds were probed with solid phase micro extraction (SPME) needle and the thermally desorbed species were analyzed for targeted substances.

With this distinctive sampling procedure, which generally favours volatiles over semi-volatile compounds, it was possible to obtain characteristic chemical profiles in terms of GC/MS data, which makes it possible to identify as well as quantitate (on a relative basis) many compounds in a single run. Several classes of volatile organic compounds were identified, including aldehydes, ketones, alcohols, and terpenes. Good inter-run reproducibility of concentration profiles was found, which speaks in favour of the robustness of the method.

The compounds of special interest for unequivocal identification and tracing the origin, to represent characteristic grazing pastures and were clearly distinguished in the GC/MS data. To distinguish with other compounds that are linked more to specific manufacturing practices these represent a unique chemical profile for distinguishing the original cheeses from possible surrogates.

The European dairy industry at the turning point

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In the developed countries, dairy industry is one of the most important economic activities in the agro-food chain, characterised by extreme vertical integration, limited international trade and protectionist intervening of the state. After eastern-ward enlargement, the European Union (EU) further strengthens its dominant role in the world production of milk and will progressively reform common market organisations for milk and milk products. The environment in which the dairy processing sector in the EU-25 will be operating in the future is the one in which milk supply is likely at least in the short term - through quotas, while greater trade liberalisation and consequently prices are likely to occur only in the longer term. However, changing agricultural policy measures will be only one segment of changes of the economic environment affecting the dairy markets. The effects on the side of production factors demand conditions, farm structures and rivalries will be of importance, as they determine the future competitiveness not only of the European dairy sector of each company or agricultural holding forming part of this chain. This paper tries to point out individual driving forces and trends that will affect the development of dairy sector in the next ten years in general in the European Union, with a special emphasis on the agreed and foreseen changes in EU dairy policy.

The main hypothesis is that the EU dairy sector will be in the coming years subject to extreme pressures for changes, which will affect the structure and functioning of the entire chain. Also at enlargement, the EU will face high costs of basic factors such as labour and land in comparison with the main competitors. Home market will be large, but saturated and extremely demanding. Competition strengthens largely because of powerful retailers and food service chain, and increased intra-competition on expanded single market. The enlargement will on the one hand, temporarily increase the pressures for the present 15 Member States as they will be able to sell on eastern markets, on the other hand, the situation will become tougher for the producers in the Central and Eastern Europe. The scale will become a necessity for all dairy companies, except for a few small players in very niche markets. Beside the volume and constant supply, a lower price of raw milk can constitute a major competitive advantage for dairy companies. EU dairy policy will be forced to change, however, the medium-term perspective, the political cost of quota abolishing would be too high and EU policymakers decided in 2003 for a gradual liberalisation of the sector. Some compensation for the reform is necessary from the political-economy perspective; it should be de-coupled and connected with providing of the public goods. However, the power and the willingness of the policy makers to overcome the market forces in the dairy, as was the case in the past, has been diminishing.

The further liberalisation of the dairy sector will require major changes at the farm level. Greater market awareness and market-led innovations in the dairy processing industry will also be needed. A move to more competitive environment will require a significant shifting of priorities within the companies and change in their business culture. This will call for an increased focus on innovation, new product development and marketing: an accelerated development of greater value added products, such as dairy ingredients and functional food, and the development of the international market.

The consequences of changing conditions of the European dairy sector for the strategies of dairy companies

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In recent years, the conditions for the European dairy industry have changed dramatically. The following changes seem to have major importance for the strategies of dairy companies:

- ▶ **The realisation of the Mid Term Review:** Important details are the extension of the quota regime until 2014/15, the increase of quotas by 1.5 % after 2006, the reduction of the intervention prices and the introduction of direct payments starting in 2004/05.
- ▶ **The EU-extension to the CEEC-countries:** In May 2004, the EU will be extended and 10 new member countries will join the EU.
- ▶ **Structural change in the dairy industry continues.** In some European countries like the Netherlands and Denmark/Sweden very large dairy enterprises with high output and market power and the chance to perform a strategy of cost leadership have been established. In many other countries still many small size dairies exist.
- ▶ **Concentration and internationalisation of the food retail trade** accelerated in recent years. Furthermore, private labels and the discount trade gain market shares.

Based on these changes, the **following strategies of the dairy industry** will be discussed:

- ▶ **Cost leadership:** Investigations show large differences in the cost of production between dairy enterprises. Differences in factor costs but also the size of the processing unit are very important. It is expected that in the future the production of standard dairy products will be a domain of large companies able to perform cost leadership.
- ▶ **Differentiation strategy:** This strategy has the advantage that the dairy can escape the problem of exchangeability and the strong focus on low prices. Branding, product innovation and promotion are the most important measures to differentiate dairy products. However, these goals are very costly and afford a high budget for marketing. For many SME's of the dairy sector a differentiation strategy therefore is no perspective.
- ▶ **Focus strategy:** This means that the dairy enterprise serves one market niche only. This might be even for SME's profitable, if they are able to offer suitable products like biological dairy products and special cheese. If a dairy enterprise has a good standing and a sufficient market potential in the home region such a strategy could even in the long run guarantee the survival of SME's in competition to large enterprises.
- ▶ **Internationalisation:** An internationalisation strategy of dairy enterprises can have many advantages. However, to optimise this strategy, many details like the selection of the optimal markets, the market entry strategy, the optimal time for the market entrance and the selection of suitable marketing measures have to be clarified.

It will be shown, to what extent the relevance and the focus of the strategies mentioned has to be changed and which strategic adjustments have to be performed by the dairy enterprises.

Nomen est omen

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When Slovenia will enter EU, categories of milk put on market will have to be equal to those found in other EU countries. Therefore some of products traditionally known to consumers will stay unchanged while some will be slightly changed. Producers will not be completely free when choosing the names for such products since national legislation will have to follow the EU legislation. Due to linguistic peculiarities meaning of the terms used for the same product in different official EU languages are not exactly the same. The Slovene legislators faced the problem which word or expression they should have used in order to satisfy the Regulation. The research was done with the goal to find out what different possible expression mean to consumers. This would help legislators to choose such an expression which would help to either reinforce or change the existing beliefs and attitudes about products.

EU accession - economic implications for the Slovenian dairy chain

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The approaching Slovenia's accession to the European Union in 2004 will certainly be one of the biggest challenges the country has experienced so far. The integration will impose substantial changes of the economic environment for the Slovenian agro-food sectors and the dairy chain will certainly be among the most affected. The research presented in the paper applies "vertical perspective" of the accession effects evaluation developing the Computable General Equilibrium model of the Slovenian agriculture and food industry "GEMSAFT". Modelling scenarios have been developed simulating the expected macroeconomic and agro-policy environment after the accession with special focus in the dairy chain. Obtained results provide us with additional insight to the integration process and economic implications that so far has not received adequate research attention.

The model "GEMSAFT" developed for this study follows the standard neoclassical formulation and structure however it includes some special features (explicit modelling of subsidies in agro-food sector, factor mobility restrictions two modelled regions of the rest of the world). Economic linkages are defined with a set of non-linear equations describing the behaviour of the identified agents and technological, budget and institutional constraints they are facing. The model is calibrated using originally estimated social accounting matrix (SAM) of Slovenia for the year 2000 with 29 production/commodity sectors. Ten of them are agricultural twelve food processing the rest of the economy is aggregated into seven accounts. Experiments with the "GEMSAFT" model involved comparative static simulation of the post accession economic environment in Slovenia, in particular: custom union with the EU-25, import and export price changes application "CAP" financial package and harmonisation of the export subsidies level for food products.

Simulations of the Slovenian EU accession with the "GEMSAFT" revealed some apparent results. Effects on production and value added in agriculture are rather small with some sectoral exceptions, whereas the food processing sectors show decrease of both indicators. Furthermore, the results show that both segments of the Slovene dairy chain are clearly negatively affected by the accession. Value added in the dairy sector will decrease by more than two percent whereas in the milk processing industry contribution will be down by 4.6 %. Foreign trade reorientation is another evident effect of the accession, especially on the export side. Rather low and regionally concentrated foreign sales of dairy products are expected to decrease by almost 20 % whereas import to Slovenia will increase by seven percent.

Slovenian dairy chain will face substantial changes of the economic environment after the accession to the European Union. Economic effects are rather unfavourable and explicit vertical transmissions between the chain components are evident. Due to the economic and political significance of the sector immediate responses are required by all included parties: farmers, industry and government to prevent even more negative effects of the accession.

System of milk quota implementation in Slovenia

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In accession negotiations Slovenia has gained 467.063 tonnes of quota for supplying and 93.361 tonnes of quota for indirect sale on the farm on the basis of statistical data from 2000. Besides it also gained a reserve in the amount of 16.214 tonnes for the year 2006. These quotas will have to be allocated among individual owners for the first quota year 2005/06 (quota year from April 1 until March 31 next year), when the system should already be functioning. Slovenia decided to perform test quota year in the year 2004/05. In this year, all groups which will be put to the quota system will be adequately prepared. In the test quota year the computer application will be tested and potential mistakes that could complicate normal functioning of such a system can be removed in the first quota year.

The Regulation which shall define precise rules of the milk quota system functioning is prepared by the Ministry of Agriculture, Forestry and Food (hereinafter referred to as: the Ministry). The Regulation is expected to be published until the end of this year. Currently there is a discussion to the proposals prepared by the working group within the Ministry.

The implementation of the milk quota system was delegated to the Agency of the Republic of Slovenia for Agricultural Markets and Rural Development (hereinafter referred to as: the Agency). An important role within the system will be played by the purchasers of milk who will perform administrative procedures, relating to the quota for the supply of dairies. Control will be exercised by the Agency and the Inspectorate of the Republic of Slovenia for Agriculture, Forestry, Hunting and Fisheries.

Pursuant to the current proposals the Agency will implement the allocation of national quotas to individual owners, the following procedures will be implemented during the whole quota year:

- ▶ collection, management and control of milk registers on supplying dairies with milk and sale of milk and milk products on agricultural farms,
- ▶ quota conversion (temporary - within the quota year - and permanent),
- ▶ quota transfers (only permanent transfers are foreseen for Slovenia),
- ▶ registration of purchasers and control over their work,
- ▶ formation of national reserve and allocation of quotas from the national reserve,
- ▶ calculation and collection of taxes for the overrun of quotas,
- ▶ reporting to Brussels and the Ministry.

For all procedures there will be a computer application set up at the Agency, which will ensure the functioning of the whole system. The purchasers who will perform a part of administrative procedures will also be committed to this application.

For the second year the Agency is collecting records on produced and sold and/or delivered quantities of milk (for the years 2001/02 and 2002/03). These records will be collected every year and will serve as a basis for the allocation of individual quotas. In the reference year 2002/03 there were 19.817 agricultural holdings included in the system of reporting on milk quotas.