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QUANTITATIVE CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* BEHAVIOUR IN MILK IN RELATION TO ARTISANAL EWES` CHEESE FERMENTATION

Ľ. Valík, A. Medveďová, D. Liptáková

Slovak University of Technology, Faculty of Chemical and Food Technology, Department of Nutrition and Food Assessment, Slovakia

A quantitative growth analysis of *Staphylococcus aureus* was carried out in milk fermented with mesophilic mixed-strain culture mostly in relation to the conditions prevailing during artisanal ewes` lump cheese production originally carried out at farm level in mountains areas of Slovakia. Both temperature and initial volume of the culture had a dramatic effect on the behaviour of the *S. aureus* strain under study. Depending on the condition, the growth, reaching maximal population density (N_{max}) and strong inhibition of *S. aureus* were observed. Regression analysis of the results enabled us to explain the relationships among parameters as growth/death rate, lag-phase and N_{max}-N_0 and the temperature and initial culture density on the other hand. As an example, the rate of *S. aureus* inhibition (r_{inh}) was described by following equation: $r_{inh} = -0.1302+0.02325 \times T-0.000975 \times T^2-0.00001 \times T^2 \times V_0^2$ (R=0.965). The most useful for good manufacturing practice was also the fact that *S. aureus* increased its number mostly only within 1 log and did not reached 10^6 CFU/mL during our model fermentation.

The knowledge found in this study regarding quantitative behaviour of *S. aureus* including the information on its growth/inhibition at selected environmental factors (temperature, inoculum size and pH) is essential for the improvement of fermentation process, quality and safety of artisanal ewes´ cheese production used as a raw material for industrial Bryndza-cheese production.

This work was supported by the contract No. APVV-20-005605 and VEGA project No. 1/0126/09.

FATE OF *STAPHYLOCOCCUS AUREUS* IN THE PRESENCE OF LACTIC ACID BACTERIA IN MILK AND EWES´ LUMP CHEESES

A. Medveďová, Ľ. Valík, D. Liptáková, A. Hudecová

Slovak University of Technology, Faculty of Chemical and Food Technology, Department of Nutrition and Food Assessment, Slovakia

The preparation of ewes´ lump cheese from raw or pasteurized ewes´ milk, especially due to the activity of lactic acid bacteria has been known in Slovakia for a long time. In cases of lower lactic acid bacteria activity, pathogen microorganisms including *Staphylococcus aureus* may breed excessively, produce enterotoxins (SE) and the SE intoxication may break out, consecutively. The encouragement of the acidification process by the starters is profitable to use with the respect to the quality of the product. Therefore, the aim of our work was to quantitatively characterize the potential of lactic acid bacteria to keep the growth of *S. aureus* under the control in milk and ewes´ cheeses prepared in laboratory conditions with addition of starter cultures.

Based on LAB and *S. aureus* co-culture trials at 21 °C, as the recommended temperature for appropriate process of cheese acidification, the necessary initial number of starter culture were described. The up growth of *S. aureus* in milk co-culture with Fresco or Acidko culture was related with initial number of LAB by the following relations: $N_{max\ STA} - N_{0\ STA} = -0.520*N_{0\ Fr} + 4.057$ ($R^2 = 0.986$) or $N_{max\ STA} - N_{0\ STA} = -1.816*N_{0\ A} + 9.288$ ($R^2 = 0.866$), respectively.

With respect to the inhibitive potential of Fresco and Acidko culture, the ewes´ lump cheeses with their addition were prepared in laboratory conditions. Similarly, the adequate addition of both lactic acid bacteria cultures during cheese manufacture and the following pH decrease on the levels lower than 5.0 for 1 to 2 d were able to inhibit the growth of *S. aureus* on concentrations lower than 10^4 CFU/g required by European Union legislation.

This work was supported by the contract No. APVV-20-005605 and VEGA project No. 1/0126/09.

Lactococcus lactis modulates *Staphylococcus aureus* virulence in cheese matrix

Marina Cretenet[1], Sergine Even[1],Cathy Charlier[1], Sébastien Nouaille[2], Muriel Cocaign-Bousquet[2], Pascal Loubière[2], Yves Le Loir[1]
[1] UMR 1253 INRA-Agrocampus, 85 rue de Saint Brieuc, 35042 Rennes Cedex, France.
[2] LISBP, UMR CNRS 5504, UMR INRA 792, INSA, 135 Avenue de Rangueil, 31077 Toulouse Cedex 4, France.
+33 2 23 48 59 44
marina.cretenet@rennes.inra.fr

Oral presentation

Lactococcus lactis is widely used in dairy products and contributes to curd formation and organoleptic properties. *L. lactis* is also able to inhibit undesired bacteria such as *Staphylococcus aureus,* an opportunist pathogen that remains a major cause of food poisonings, in dairy products. Some strains can produce enterotoxins whose ingestion causes the staphylococcal food poisoning symptoms. Our goal is to better understand the mechanisms involved in the interactions between *L. lactis* and *S. aureus* to improve food safety.

A transcriptomic approach was thus developed to investigate the effect of *L. lactis* on *S. aureus* gene expression profile, including genes encoding enterotoxins and other virulence factors. Conversely, the effect of *S. aureus* on the expression of *L. lactis* genes of technological interest was analysed. Food fermentations are a dynamic and complex process in which microorganisms are subjected to a particular physico-chemical environment and to multiple stress conditions and interactions that vary from planktonic model cultures. Therefore, the microbial interactions were analysed directly in a cheese matrix model, enabling the dynamics of the *in situ* response to be established.

The most striking results were observed during the post-exponential growth phase of both microorganisms where the presence of *L. lactis* impaired the induction of several staphylococcal virulence factors and modulated enterotoxin gene expression. On the opposite, the transcriptomic profile of *L. lactis* was only moderately affected. This result clearly underlines the complexity of *L. lactis* antagonistic potential against *S. aureus*. This approach opens new perspectives for a better control of the interactions between microbial species in cheese matrix.

This work was supported by «ANR- French National Research Agency» and by «CNIEL-the French National Interprofessional Centre of the Dairy Economy».

Diversity of *Staphylococcus aureus* isolates from bovine milk with a high somatic cell count.

J.L.W. Rademaker, Y. Xiao, J.D. Hoolwerf, P.H.M. Savelkoul, M.H.J. Wells-Bennik
NIZO food research, Kernhemseweg 2, 6718 ZB, Ede, The Netherlands (marjon.wells-bennik@nizo.nl)

Introduction

Staphylococcus aureus is a cause of bovine mastitis and affects milk production and animal welfare. This bacterium can also cause foodborne disease, but only when present in high numbers in food due to toxin production. The organism is also an important source of hospital- and community-acquired infections, and is therefore a concern for public health. *St. aureus* isolates are widespread, but methicillin-resistant variants (MRSA) are emerging. The aim of this study was to determine the characteristics of *St. aureus* isolated from bovine milk with high somatic counts.

Methodology

The presence and diversity of *St. aureus* was investigated in samples of raw bovine tank milk from Dutch dairy farms with suspected mastitis cases, based on a high somatic cell count (SCC >400.000 cells /ml). *St. aureus* strains were isolated from several hundred random samples using selective Baird-Parker medium. The identity of the isolates was confirmed using a *S. aureus*-specific real-time PCR-method. All isolates were evaluated for the presence of the *mecA* methicillin resistance gene using two different PCR tests. To assess their diversity, *spa* typing was performed. This high resolution method is based on DNA sequence variation in the protein A gene.

Results and discussion

74 *St. aureus* strains were isolated from raw milk with high-SCC. The *mecA* gene was not present in any of these strains, thus no MRSA isolates were present. The diversity analysis showed 20 different *spa* types. These will be discussed in relation to those of major clonal lineages associated with human origin, antibiotic resistance profiles and animal to human transmission. Overall, typing patters of strains found in milk were distinct from common hospital-associated *St. aureus* strains.

Contribution to the advancement of dairy-related knowledge

St. aureus isolates in high-SSC raw milk showed a wide genetic diversity and the *mecA* gene was absent from these strains.

Identification of key technological factors in the fight against *Staphylococcus aureus* during cheese manufacture

Authors : Manon Duquenne, Isabelle Bataillon, Marina Aigle, Claire Darrigo, Jacques Antoine Hennekinne, Isabelle Papinaud, Meriam Kanoun, Chizuko Sakamoto, Véronique Deperrois-Lafarge, Agnès Delacroix-Buchet, Marielle Bouix

Abstract

Staphylococcus aureus is reported in France as the most frequent pathogen involved in food-borne diseases associated with milk and dairy products. Food poisoning results from ingestion of staphylococcal enterotoxins preformed in food by enterotoxigenic strains of *S. aureus*. In order to identify key technological parameters affecting *S. aureus* growth, enterotoxin gene expression and production during cheese manufacture, we studied the impact of semi-hard cheese process parameters on *S. aureus* behavior in controlled environmental conditions. Parameters were chosen according to i) results of CNIEL survey on practices amongst semi-hard cheese manufacturers and ii) known environmental conditions that could induce or inhibit *S. aureus* development. With Taguchi experimental approach, the effect of the five more variable technological parameters between cheese manufacturers was studied on the behavior of four *S. aureus* strains isolated from cheese and producing enterotoxin A, B, C or D. During every cheese manufacture (milk inoculated with *S. aureus* at 10^3 CFU/ml), *S. aureus* population reaches a value superior to 10^5 CFU/g of cheese four hours after molding. In all cheeses, only enterotoxin D is detected (consistent result according to observations in dairy industry) in very low and variable quantities depending on studied parameters with a maximum of 0.06 ng/g of cheese four hours after molding. This work also shows enterotoxin D production seems to be correlated with an early gene expression during cheese processing (less than 6 hours after milk renneting). Finally, the Taguchi experimental approach analysis points out milk maturation temperature is a key technological parameter for enterotoxin D production during semi-hard cheese process. A second experimental approach will allow us to understand the impact of this parameter on enterotoxin D gene expression better.

Inhibition of Biofilm Formation of *Listeria monocytogenes* by □-Casein Macropeptide

*Authors: Hyun Sun Yun[1], Younghoon Kim[3], Jin Lee[1], Sejong Oh[2], and Sae Hun Kim[1]**

[1]Division of Food Bioscience & Technology, Korea University, Seoul 136-701, Korea

[2]Department of Animal Science, Chonnam National University, Gwangju 500-757, Korea

[3]Artie McFerrin Department of Chemical Engineering, Texas A&M University, College Station, TX 77843-3122, USA

Listeria monocytogenes has the ability to attach to various surfaces and form the biofilm. Several reports have shown that biofilm produced by *L. monocytogenes* are more resistant to environmental changes and cleaning treatments than planktonic growth mode. Therefore, new inhibitors with the potential to remove mature biofilms are needed.

Our objective was to examine the capability of □-casein macropeptide (CMP) inhibit the biofilm formation of *L. monocytogenes*. Related to this factor was analyzed with two dimensional electrophoresis (2-DE).

Based on various conditions, the biofilm formation of *L. monocytogenes* strains on the polyvinylchloride (PVC) microtiter plates were indirectly assessed by staining with 0.1% crystal violet. On the selected strains, inhibition of biofilm formation by CMP (0.1, 0.2 or 0.4 mg/ml) was determined as above methods. In addition, the inhibition factors of GMP were also analyzed by 2-DE.

In the PVC microtiter plate assays, 12 strains *L. monocytogenes* exhibited biofilm formation. And the inhibition rate of *L. monocytogenes* strains biofilm was the lowest in Modified Welshimer's Broth (MWB) containing 0.4 mg/ml of CMP. Five proteins, which exhibited lower levels of expression in MWB containing 0.4 mg/ml of CMP.

This property could contribute to understanding specific mechanisms within bacterial communities and lead to the development of novel and food-grade adjuncts for microbial biofilm control.

Risk-based approach for microbial food safety in dairy industry. Application to *Listeria monocytogenes* in soft cheese made from pasteurized milk.

Dr. Fanny Tenenhaus-Aziza; Centre National Interprofessionnel de l'Economie Laitière (CNIEL) France

Advances in the quantitative risk assessment allow using this approach for food microbiological safety. Through the example of *Listeria monocytogenes* in soft cheese pasteurized milk, the objective of the study was to validate this approach as a tool for controlling microbiological hazards in food.

Based on control plans coming from the French dairy industry and a bibliographic synthesis of all the elements that can now be integrated into a quantitative risk assessment, we proposed a complete model, estimating the risk of listeriosis at the moment of consumption, taking into account the entire manufacturing process and potential sources of contamination. From pasteurization to consumption, the amplification of a initial contamination of the process environment by *Listeria monocytogenes* is simulated, over time, in space and between products, accounting for the impact of control measures, such as hygienic operations and sampling frame. A sensitivity analysis of the model allows for the identification of major parameters contributing to the risk and the optimization of preventive and corrective measures.

This model, which can be adapted to other species and processes, concretely illustrates the interest of the quantitative risk assessment in food safety.

Key-words : Modelling, Microbiological quantitative risk assessment, cheese process, contamination by the environment.

Study on spore forming thermophilic and mesophilic *Bacillus* in industrial and traditional cream in Urmia

*Negin Noori [*1], Seyyed Ali Ghaemi[2], Guti Karim[1]*

1- *Department of food hygiene, Faculty of veterinary Medicine, University of Tehran, Tehran, Iran.*
2- *Graduated student of Veterinary Medicine, Islamic AZAD University, Urmia Branch, Faculty of Veterinary Medicine.*

- *Corresponding author: Negin Noori, email: nnoori.ut.ac.ir*
- *Adress: University of Tehran, Faculty of veterinary Medicine, P.o.Box; 14155-6453- Tehran- Iran.*

In samples of cream obtained from Urmia market and transferred to the laboratory in cold condition. One gram of cream was weighted and transferred to the test tubes containing 9 ml of physiological serum. The test tubes were shaker to homogenize the sample. The test tubes were heated over 80˚ C water bath along with a control to destroy the vegetative forms of bacterial. The samples were diluted and 3 dilutions from each sample of industrial and traditional were cultured using starch milk agar (SMA). Duplicate cultures were incubated in thermophilic and mesophilic incubators. After 3 days plates were counted and from different colonies smears were prepared and Gram stained. The colonies were pured and carried out for biochemical tests. The results showed that *Bacillus Licheniformis* is the *Bacillus* in cream samples and *B.subtilis*, *B.cereus* and *B.coagolans* consisted the *Bacillus* flora in cream samples respectively.

Average number of mesophilic spores in traditional way 2.904×10^4 and industrial way 1.7×10^4 and average number of thermophilic spores in traditional way 3.014×10^4 and industrial way 6.25×10^2, traditional way in total 3.2054×10^4 and industrial way 1.7655×10^4.

The number of isolated spores from industrial creams in mesophilic condition: *B.cereus* 13, *Bacillus Licheniformis* 18, *B.subtilis* 11, unknown *Bacillus* 8 and *B.coagolans* not seen and the number of isolated spores from industrial creams in thermophilic condition: *B.coagolans* 10, *Bacillus Licheniformis* 17, *B.subtilis 9 and unknown Bacillus* 14 and *B.cereus* not seen.

The number of isolated spores from traditional creams in mesophilic condition: *B.cereus* 17, *B.Licheniformis* 17, *B.subtilis* 10, unknown *Bacillus 6* and *B.coagolans* not seen and the number of isolated spores from traditional creams in thermophilic condition: *B.coagolans* 5, *B.Licheniformis* 20, *B.subtilis 17* and unknown *Bacillus* 8 and *B.cereus* not seen.

Enteric viruses in raw milk I: characterization of milk components in recovery efficiency

M.Yavarmanesh,M.Abbaszadeghan, M.B.Habibi, S.A.Mortazavi, M.R.Nasiri, M.R.Basami

The objective of this study was to characterize the role of milk components in the recovery of enteric viruses. In order to evaluate the impact of various components of milk four model milk solutions were constituted by adding lactose, whey protein, casein and fat indifferent combinations. Each model solution was spiked with six levels of MS_2 coli phage (48, 4.8×10^2, 4.8×10^3, 4.8×10^4, 4.8×10^5, 4.8×10^6pfu/ml).The soluble and insoluble components were separated by centrifuging at 40000g and viruses were enumerated by using double agar layer (DAL) technique. In case of samples spiked with low numbers of MS_2 (less than 4.8×10^5 pfu/ml), components did not have any impact on recovery, but this confirmed in higher spiked. Higher recovery efficiency was achieved in all of the precipitation from all models, except in the model D (lactose + whey protein + casein + fat). The highest number of coliphage were recovered from the precipitation of model C (lactose + whey protein + casein). In general, higher number of MS_2 was detected in the supernatants of all of the models. The dry matter contents of milk are related with the recovery efficiency of virus. Based on the results it can be concluded that the best recovery of enteric viruses in raw milk could be achieved by eliminating the milk, whey and casein (by centrifugation at 40000g) before sample analyses.

Keywords: Enteric virus, raw milk, Milk components, Recovery efficiency

Enteric viruses in raw milk II: Evaluation the impact of milk components on RNA extraction

M.Yavarmanesh,M.Abbaszadeghan, M.B.Habibi, S.A.Mortazavi, M.R.Nasiri, M.R.Basami

In order to evaluate the impact of milk components in viral RNA extraction, four model milk solutions were constituted by adding, lactose, whey protein, casein and fat in different combinations. Each model solution was spiked with five levels of MS2 coli phage(1.3×10^{4} ,1.3×10^{2} ,1.3,1.3×10^{-2} and 1.3×10^{-4}pfu/ml).Phenol-guanidine thiocyanat-chloroform method was then used as a basic protocol and RNA extraction was measured by nanodrop spectrophotometer based on ng/µl. The results showed that casein and whey protein had the highest inhibition on RNA extraction, especially when number of MS2 coliphage was less than 1.3pfu/ml. In the last model (L+W+C+F) which has also consisted of milk fat, we had the most RNA yield. The dry matter content of milk was closely correlated with the RNA extraction ($R^{2:}$0.93).According to the results, it can be concluded that, milk fat is the most effective component in facilitating of RNA extraction, so that the best protocol for RNA extraction could be achieved via elimination of milk whey protein and casein by centrifugation at 40000×g for 60min..Milk fat will be recombined to supernatant and homogenized before molecular experiments.

Keywords: Enteric virus, Raw milk, Milk components, RNA extraction

Effect of storage and preservation on bacterial count determined by automated flow cytometry in bulk tank goat milk

D. Sierra[1], A. Sánchez[2], A. Contreras[2], C. Luengo[1], J.C. Corrales[2], C. de la Fe[2], I. Guirao[2], C.T. Morales[1], and C. Gonzalo[3]

[1]Laboratorio Agroalimentario y de Sanidad Animal, Consejería de Agricultura y Agua, Comunidad Autónoma de la Región de Murcia, El Palmar, 30120 Murcia, Spain.
[2]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad de Murcia, 30071 Murcia, Spain.
[3]Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, *24071 León, Spain.*

This study was designed to evaluate the effects of different analytical conditions on automated total bacterial count (TBC) determinations made in goat's bulk tank milk. To this end, 5,530 individual bacterial counts (IBC) were obtained in 2,765 aliquots taken from 35 bulk tank milk samples from 35 Murciano-Granadina goat herds, using an automated flow cytometry method (BactoScan FC, Foss Electric, Hillerød, Denmark). The conditions tested were storage temperature (refrigeration at 4ºC and 10ºC, or freezing at -20ºC), the use of a preservative (no preservative, NP; azidiol, AZ; or bronopol, BR) and the age of the milk samples (storage times at 4ºC: from 0 h to 5 d for NP; and from 0 h to 22 d for AZ and BR; storage times at 10ºC: from 24 h to 2 d for NP and from 24 h to 22 for AZ and BR; storage times at -20ºC: from 24 h to 22 d for NP, AZ and BR). Significant effects on logIBC variation were shown by: the bulk tank milk sample, preservative, storage temperature, interaction preservative x storage temperature, and milk age within the interaction preservative x storage temperature. As expected, highest IBC were obtained in the NP milk samples. In preserved samples, highest IBC were obtained for AZ, and lowest counts were obtained in samples preserved with BR. Because of the variation in IBC recorded in BR preserved samples, we recommend this preservative should not be used for TBC determinations using the automated FC method. NP samples showed significantly higher IBC, also invalidating this analytical condition for TBC analyses. The practical implications of our findings are that goat's milk samples preserved with AZ and stored at 4ºC, 10ºC and -20ºC are appropriate for TBC by the BactoScan FC method for as long as 11, 9 and 22 days post-collection, respectively.

Kinetik der thermischen Inaktivierung von Flaviviren in Milch

Dipl. Agr.-biol. R. Saier, Prof. J. Hinrichs. Institut für Lebensmittelwissenschaft und Biotechnologie, Fg. Lebensmittel tierischer Herkunft, Universität Hohenheim

Die Frühsommermeningoencephalitis (FSME) ist die bedeutendste durch Zecken übertragene Viruserkrankung Europas und eine klassische Zoonose. Der Auslöser der FSME gehört zum Genus *Flavivirus* in der Familie der *Flaviviridae* und wird im Allgemeinen direkt durch den Stich einer infizierten Zecke (*Ixodes ricinus, Ixodes persulcatus*) übertragen. Dies gilt, abgesehen vom Menschen, ebenso für milchliefernde Nutztiere. Bei ihnen können während der Infektion über einen Zeitraum von bis zu 10 Tagen FSME-Viren mit der Milch ausgeschieden werden. Im Sommer 2008 infizierten sich in Österreich mehrere Personen beim Verzehr von Rohmilch-Ziegenfrischkäse mit FSME-Viren. Die Ausscheidung der Viren mit der Milch während des Infektionsgeschehens stellt somit eine potentielle Gefahr beim Konsum von Milch und der daraus hergestellten, insbesondere nicht pasteurisierten Produkte dar.

Bei der Untersuchung von Ziegenherden im süddeutschen Raum zum Vorkommen von Antikörpern gegen FSME-Viren wurden mit einem kommerziellen ELISA-Kit (Immunozym FSME IgG All Species, PROGEN BIOTECHNIK) Seroprävalenzen zwischen 0 und 1,6 % festgestellt. Die Versuche zur Thermoresistenz des in Süddeutschland auftretenden FSME-Virusstamm Hypr wurden in Milch vorgenommen. Dazu wurde rohe Magermilch mit Viren in einer Konzentration von 4×10^7 $KID_{50}\,ml^{-1}$ (kulturinfektiöse Dosis) versetzt und in den bei der Milcherhitzung relevanten Bereichen im Thermocycler erhitzt. Im Zellkulturtest auf 'porcine stable kidney' Zellen wurde danach die Infektiosität durch Auftreten eines cytopathischen Effekts (CPE) bewertet und die kulturinfektiöse Dosis berechnet. Die ermittelten D-Werte bei Temperaturen von 65, 70, 75 bzw. 80 °C sind ca. 40, 10, 9 bzw. 7 s.
Durch die kombinierte Beschreibung von FSME-Antikörperseroprävalenzen bei milchliefernden Nutztieren und des Inaktivierungsverhaltens von FSME-Viren während der thermischen Behandlung von Milch soll eine Empfehlung zur Verarbeitung von Milch aus für das Auftreten von FSME bekannten Gebieten abgeleitet werden.

Microbiological Quality and Composition Properties of Selected Cow's Milk Produced in Balikesir Region

Mikail Arslan, Reyhan Irkin, Adem Kabasakal

Balikesir University, Susurluk Milk and Meat Industry College, Susurluk TR10600, Balikesir, Turkiye

There is an increasing demand by the dairy industry for high quality and free from microorganism raw milk. Good quality milk is important for a profitable dairy industry. Present study focuses on to compare the sanitary quality, composition properties and average daily milk production quantity of milking herds according to their size scales. Milk was sampled from the 40 different Holstein cow's herds located within an 30 km radius of the laboratory in the Susurluk, Balikesir, Bandirma and Mustafakemalpasa regions. The cow's herds were seperated in four groups (n=4×10) according to their size scales; 1-5, 5-15, 15-40, > 40 cows/herd. Raw milk from each herd was sampled throughout during 3-5 months of lactation stage. Lactic acid (SH°), pH, fat%, crude protein%, lactose%, total solid% contents and total viable bacteria, feacal coliform, *E.coli*, moulds and yeasts, *B.cereus*, *Pseudomonas* spp., somatic cell counts were researched and evaluated statistically. In >40 cows herd total viable counts ($p< 0.05$), *E.coli*, Mould and yeasts counts and average daily milk production yield ($p<0.01$) different from other small and medium size scales herds. *B.cereus* and *Pseudomonas* spp. were not detected in the milk samples. As a result, the chemical composition of cows milk indicates that produced milk in local dairy industry containe a rich source of nutrients. Nevertheless, the presence of some indicator bacteria in small holder dairy herds may lead to a hazard against public health. Therefore it is recommended that hygienic practises and regulations such as implementation of HACCP should be introduced to the small holder dairy farms for production of high quality and safety milk. In dairy industry, another important factor is to maintain transition to the > 40 cows herds farms from small herds.

K*ey words*: *Microbial quality raw milk, dairy milking herds, milk composition*

CoolChurn® -
the first selfchilling milk churn

Since 1999 Cool-System KEG GmbH acts as the developer and the patent holder worldwide for sorption cooler for cooling of liquids with content from 5,0l to 60,0l. The selfchilling keg for beer and wine, CoolKeg, had been presented in 2000. The own CoolKeg production line with an annual capacity of 35.000 - 50.000 CoolKegs / CoolChurns has been put into operation in Burkau near Dresden in 2003 (see picture). More than 100.000 CoolKegs had been supplied worldwide in the past 5 years. In 2009 Cool-System KEG GmbH now presents the selfchilling milk churn – CoolChurn.

CoolChurn® - Specifications

- Cooling system without ice, energy or water supply
- Cooling of the milk to 5-8°C within 2-3 hours
- Cooling „at the push of a button“: the farmer starts the cooling of the milk if required
- CoolChurn content: 18,0l
- The Temperature will be kept for at least 12 hours
- Physical cooling with zeolite-water-adsorption – no danger for the user (farmer); environmentally friendly; returnable milk churn

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CoolChurn® - Advantages

Application areas:
Agricultural countries with bad infrastructure where farmers only have few milk cows and no possibility to cool the milk on location.

Logistic:
The selfchilling milk churn will be regenerated, cleaned through the dairy company and then the farmers will be provided with the CoolChurns. The peasant returns the the filled milk churn with the cooled milk after one day back and gets on the other hand a empty and regenerated CoolChurn. It is a matter of closed product circulation.

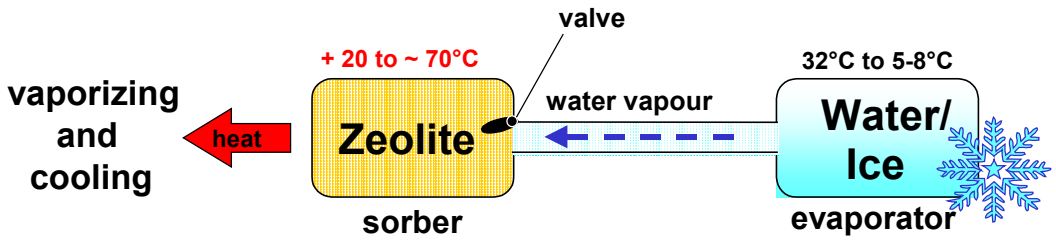
Aim and usefulness:
The microbial count of the delivered milk will be reduced by 80-90%. The usage of a cleaned locking container improves the hygiene of milk significantly.

Zeolite Cooling Technology (Zeolith/Water-Vacuum Adsorption Technology)

The essence of the cooling technique is the Zeolite/Water-Vacuum Adsorption Technology. Zeolite is a non-toxic mineral that exists in nature. In dry conditions it adsorbs large quantities of water. Under vacuum the process makes it possible to produce ice.

How does it work in the CoolChurn?

Under vacuum a reservoir of water is connected with the Zeolite over a vapour line. When somebody opens the vapour line through opening the valve by turning the lever on the top of the CoolChurn, the vapour flows from the surface of the water reservoir to the Zeolite where it will be adsorbed. The emerging vapour reacts to the Zeolite; the Zeolite gets warm and the water reservoir cools down till it freezes. This can be likened to the sudden chill people experience coming out of the sea or a pool during a cold breeze. On the other hand, the Zeolite heats up through the kinetic energy taken up with the water molecules.



functional diagram of the Zeolite/water-vacuum adsorption

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Telefon.: +49-911 9776-752

Environmentally friendly regeneration of the cooling technology

The CoolChurn will be returned to the dairy company. There the CoolChurn can be set back to its original condition that means regenerated, so that it can cool again at the push of a button.

The process is strictly physical; there is no change of substance as in chemical processes at all. All substances enclosed in the CoolChurn are absolutely environmentally friendly and non-toxic. There is no danger of poisoning or exploding.

In a continuous or batch regeneration-process the surface of the CoolChurn is heated up to 350 °C to reverse the physical process, that means to evoke the Zeolite to repulse the enclosed water. During the regeneration process the valve is closed. The closed valve opens in response to the pressure of the steam, and condensation occurs on the cool milk container.

For this regeneration process we developed different regeneration equipment, electrically or gas-driven:

Heating Collar

Heating Chamber

Continuous CoolChurn Charger

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Practical experience with process hygiene criteria referring to the safety of milk products and the reliability of certification

Ernst Jakob, René Imhof, Jörg Hummerjohann, Thomas Berger

In Switzerland, about 45 % of total cheese production is exclusively made from raw milk. Another 6 % are made from thermised milk with added fresh raw milk. The rest is produced from thermised (12 %) or pasteurized milk (36 %). Data collected within the Swiss national monitoring programme for dairy products have confirmed that hard and extra-hard raw milk cheese varieties do not pose a risk to consumers.

Unpasteurised semi-hard and soft cheese varieties are shown to be more prone to contaminations, especially to contaminations with staphylococcal enterotoxins, *Listeria monocytogenes* or Shiga toxin-producing *E. coli* (STEC). However, the frequency of contaminations strongly varies with both, type of production plant and type of cheese. Since 2007, cheese factories have to monitor coagulase-positive Staphylococci in the cheese-making process instead of testing the final products. Data collected so far from the Agroscope Liebefeld-Posieux Research Station ALP advisory service and the ALP emergency team for businesses demonstrate that potential risk of toxin formation by staphylococci in semi-hard cheese made from raw milk had been underestimated. In the following of two cases of STEC-positive cheeses ALP initiated an intensive monitoring with the products concerned on STEC, *L. monocytogenes, E. coli* and coagulase-positive Staphylococci. Samples were taken at five production stages. 100 different daily productions have been investigated within a year. In cases of unsatisfactory results for *E.coli* and coagulase-positive Staphylococci corrective measures have been implemented. STEC have been detected in one case. The process hygiene criteria proofed to be very helpful to identify weak points.

DESIGN OF A HACCP PLAN FOR THE IMPLEMENTATION OF ISO 22000:2005; A CASE STUDY FOR INDUSTRIALLY PRODUCED YOGHURT LINE

A.Demet KARAMAN*, Cavit BİRCAN

Adnan Menderes University, Faculty of Agriculture, Department of Food Engineering, 09100, Aydin, Turkey.
* demetkaraman@gmail.com

ABSTRACT:

One of the most important tasks at any dairy process is safeguarding the quality of dairy products. Ensuring the safety of a supply requires monitoring not only of the finished yoghurt, but particularly of parameters which indicate whether the key control measures in a given process are functioning correctly. Preventative measures therefore have become very important. Food Safety Management System (FSMS) (EN ISO 22000: 2005) integrates the principles of the Hazard Analysis and Critical Control Point (HACCP) system and application steps developed by the Codex Alimentarious Commission. By means of auditable requirements, it combines the HACCP plan with prerequisite programs (PRPs) and Operational PRP(s).

In the present work, the method of HACCP and ISO 22000 general principles, the necessary legislation for yoghurt and raw milk, the microbiological, chemical and physical parameters as well as the essential steps of treatment for the production of yoghurt are examined. The ISO 22000 principles were applied to the dairy plant in Aydın, Türkiye, which receives around 10,000 l of bulk milk per day, has 25 employees and processes yoghurt. Physical-chemistry and microbiological analyses analysis started with the identification and evaluation of significant health hazards of yoghurt from the farmer, treatment processes, storage, and distribution to the costumer's tap and resulted in the construction of the PRP, PRP(s) and HACCP plan for the production of yoghurt according to process flow diagram for this particular plant during 3 months. The critical control points identified include **Raw Milk Reception, Pasteurization, last filter point in the process** within the many Critical Points (CP) at the yoghurt production process. This research is a sample study and it will provide the necessary importance to actual Critical Control Points (CCP), like providing food safety in a best way by applying the conditions of ISO 22000:2005 at all food processes. Determination of these points provides an overview on how EN ISO 22000 should be managed in the dairy sector.

Key Words: Yoghurt, ISO 22000:2005, CCP.

Factors limiting quality and storage stability of E(xtended) S(helf) L(ife)-milk

Veronika Kaufmann and Ulrich Kulozik*

Technische Universität München
Chair for Food Process Engineering and Dairy Technology
85354 Freising, Germany
e-mail: veronika.kaufmann@wzw.tum.de

Foods are more and more assed by shelf life as well as organoleptic and nutritious quality. Even in the dairy industry the E(xtended) S(helf) L(ife)-milk gaines in importance. In order to obtain milk with an prolonged shelf life (up to weeks) while minimizing undesired (heat-induced) sensorical product alteration adequate processing methods (like high temperature treatment (HTT) or combination of microfiltration with subsequent pasteurisation (MF/PAST)) are used.

Until now, the ESL-technology is applied without sufficient knowledge about the effects and interactions of processing solutions on product attributes and alteration relevant to milk quality and stability. Particularly it is not known, which (microbiological, enzymatic, physico-chemical or sensorical) product changes are limiting the shelf life of ESL-milk. Towards precise process design and product optimization a fundamental investigation of these facts is required and therefore the aim of this study.

It could be shown that applying both techniques, HTT or MF/PAST, leads to a better debacterisation effect (compared to traditional pasteurisation) and in consequence the shelf life of ESL-milk is not limited by microbial growth. However, the ESL-processing doesn't result in a complete inactivation of all indigenous or bacterially released enzymes. Therefore enzymatic (particularly proteolytic and lipolytic) reactions followed by chemical degradation of decomposition products (e.g. oxidation of free fatty acids) are taking place during milk storage causing sensory quality defects and, ultimately, product spoilage. Such (rancid, putrid, cardboard and/or bitter) "off-flavours" can be observed after about 18 days of storage (at 10°C) in MF/PAST-milk. Whereas in HTT-milk lower activities of proteases and lipases can be measured (using artificial substrates like Azocasein and p-Nitrophenylcaprylate) and, thus, flavor defects are perceivable only after 30 storage days. Finally, shelf life capacity of ESL-milk is related to residual lipolytic and proteolytic actictity and therefore the processing method applied -but also to enzymatical quality of the raw product.

Determination of lead in cow's raw milk and its Correlation with Lead in water

Mehrdad Nematparvar [1]*, Behrad Radmehr[1], Jamileh Salar Amoli[2], Mehran Farhoodi Moghadam [1], Mehrdad Khoshnevis [1]

1) Faculty of Veterinary Medicine, Islamic Azad University- Karaj branch, Karaj, Iran
2) Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
(dr_mehrdad2002@yahoo. com)

Lead is a pervasive and widely distributed environmental pollutant with no beneficial biological roles .The poisoning is more common in farm ruminants, which are considered most susceptible to the toxic effects of lead. Animals get access to lead from soil, water, feed and fodder. Varied degree of lead poisoning has been reported in animals reared around different polluted areas. High lead levels in animals and human have been reported from the various parts of the world including Iran. The lead level in milk from animals exposed to environmental pollutant has serious public health concern.

In this, survey 100 samples of raw milk from 10 farms around Tehran city were collected; these samples were collected directly from cows in the farms. We also collected 10 sample of water in the same fields. After that, these samples were sent to Toxicology Research Laboratory of University of Teheran and according to the A.O.A.C method all of the samples were probing about lead. We used the Atomic Absorption Spectrophotometery for this work.

According to the result, mean of lead in milk was 264±204/19 ppb and it was more than limitation of codex 2007 (20ppb) for lead residue in the milk. Actually all of the milk samples were over the permitted level. About lead residue in water, majority of samples were close or under the codex 2007 limitation (10ppb). Results showed no correlation between lead in water and lead in cow's milk. Therefore, water can not be a source of lead in milk and we need more work on other aspects of lead residue such as weather, soil and animal's food in this area.

Keyword: Milk; Water; Lead; Correlation; Codex

POSTER

Hartmann, R., Tait, D., Haase, G.

Radioactivity in Milk - An Overview

Abstract

The poster deals with the radioactive contamination of milk.

First of all, a brief introduction is given on the exposure of humans to natural and artificial radioactivity.

The poster also describes the pathways of radionuclides into raw milk. Typical concentrations of natural and artificial radionuclides in milk are presented showing how they have been influenced by the release of nuclear fission products from different sources (the accident at the Chernobyl nuclear power plant, nuclear weapons testing). It is pointed out that nowadays the artificial radionuclides contribute less than 1% of the total radioactivity measured in milk samples. Normally, the contents of the long-lived products of nuclear fission strontium-90 and cesium-137 are less than 0.2 and 0.5 Bq* per litre of raw milk, respectively.

* Bq abbreviation for Becquerel, 1 Bq = 1 radioactive decay per second.

NANODETECT – Entwicklung von Nanosensoren für die Detektion von Qualitätsparametern in der Lebensmittelkette

Lange, A., Eden, M., Harms, C., Lanzrath, B., Hennigs, C., Hilz, H.

Anna Lange, ttz Bremerhaven, Fischkai 1, 27572 Bremerhaven

Im NANODETECT Projekt wird die Nanotechnologie zur Entwicklung bedienerfreundlicher Biosensoren eingesetzt, um die Qualitätskontrollen in der Lebensmittelindustrie deutlich zu vereinfachen. Innovative, auf biochemischen Methoden basierende Nanosensoren können für verschiedenste Anwendungen angepasst werden. Im NANODETECT-Projekt wurde Milch als Beispiel für ein flüssiges Lebensmittel gewählt. Hierbei sollen die folgenden Qualitätsparameter als Modelle dienen:

- Pathogene Mikroorganismen (Listeria monocytogenes)
- Mykotoxine (Aflatoxin M1)
- Arzneimittelrückstände (Sulphonamide und Aminoglycoside)
- Verschnitt hochwertiger Milchsorten (z.B. Nachweis von Kuhmilchprotein in Ziegenmilch)

Die zu entwickelnden Nanosensoren bauen auf immunologischen Methoden aufbauen. Dabei wird für jeden Parameter ein eigenes Modul entwickelt. Im fertigen System können die einzelnen Module kombiniert werden. Die nachzuweisenden Kontaminanten können aus einem definierten Volumen von mehreren Litern Flüssigkeit in Mikrokanälen des Sensors, auf deren Oberfläche Antikörper immobilisiert sind, spezifisch angereichert werden. Beim Durchfluss wird eine hohe Kontaktrate der Antikörper mit den Antigenen gewährleistet und bei signifikanter Zeitersparnis gegenüber üblicher Methoden eine Quantifizierung der Kontaminanten ermöglicht.

Die Projektpartner entwickeln Nachweissysteme, die die folgenden Vorteile gegenüber konventionellen Methoden aufweisen:

- Deutlich geringere Kosten
- Hohe Umsatzraten
- Schneller, quanitiativer Nachweis
- Anwenderfreundlichkeit
- Robuste Konstruktion, die dauerhafte Nutzung ermöglicht
- Sowohl für off-line- als auch für on-line Messungen einsetzbar

BIOTECON Diagnostics GmbH
Hermannswerder Haus 17 - 14473 Potsdam - Germany
Phone: +49 331 2300 200 - Fax +49 331 2300 299
www.bc-diagnostics.com

Rapid Detection of *Enterobacteriaceae* Including Identification of *Cronobacter (E. sakazakii)* in one Single Test

Matthias KIEHNE, Cordt GRÖNEWALD, Alois SCHNEIDERBAUER

Presenting Author: Alois Schneiderbauer, email: aschneiderbauer@bc-diagnostics.com
BIOTECON Diagnostics GmbH, Hermannswerder Haus 17, 14473 Potsdam, Germany,
Phone +49 331 2300-200, Fax +49 331 2300-250, Internet www.bc-diagnostics.com

Abstract:

A new real-time PCR system for the detection of all *Enterobacteriaceae* with simultaneous identification of *Cronobacter* (according EC 2073:2005) will be presented. Using this method, the time to result is less than 24 hour including a pre-enrichment step compared to up to 5 days using the ISO method. It is easy to use and can be carried out in any lab of the dairy or infant formula producing industry. It can be applied to finished products as well as to in process testing.

The test is designed to run on all relevant real-time PCR instruments and comprises all necessary reagents. The method is thoroughly validated for the use in infant formulae with and without probiotic bacteria as well as raw materials, environmental samples and other dairy products. During the validation of the method it was recognized, that most infant formula products and milk powders contain a background of inactive or non-cultivatable *Enterobacteriaceae* which leads to positive results in the PCR but cannot be confirmed by cultural methods. To overcome this discrepancy, Reagent D is applied in advance to the DNA extraction, a reagent which inactivates DNA from dead cells for PCR. The kit also includes all necessary controls such as positive and negative and an internal positive control to eliminate false negative results due to inhibition or failures (according ISO 22174:2005).